

**Sexual Conflict and Sexual Selection in the Indian
Meal Moth, *Plodia interpunctella*.**

Submitted by

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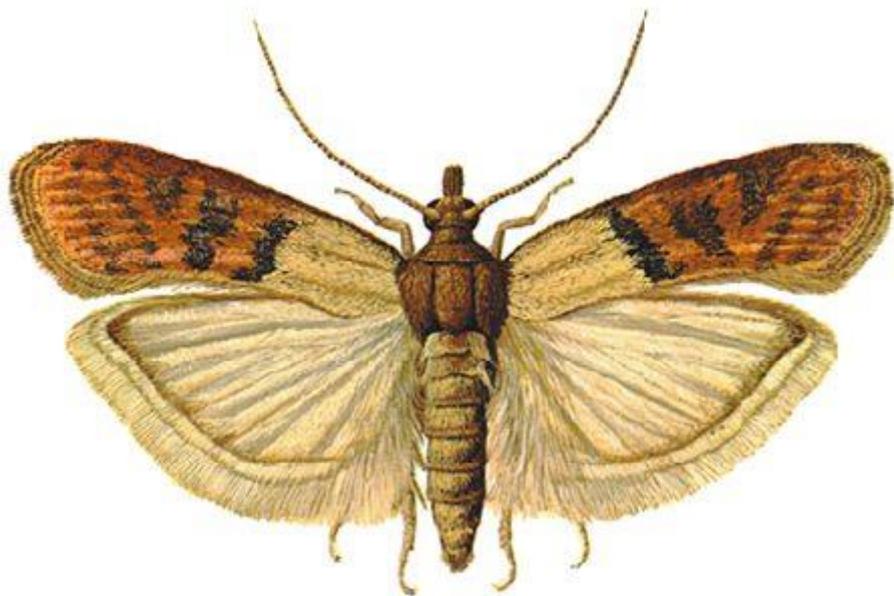
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Abstract

Sexual conflict is a powerful evolutionary force, arising due to the differing reproductive interest of the sexes. As males and females contrast in what they require to increase their own relative fitness, this creates sexual conflict. This thesis investigates sexual conflict and sexual selection in the Indian meal moth *Plodia interpunctella*, a species known to be under strong intralocus sexual conflict for shared life-history traits and fitness.

Through the use of experimental evolution, we altered the adult mating sex ratio to either male biased or female biased. This changes the selective forces between populations that may directly impact on intralocus sexual conflict. Male biased populations were found to have increased reproductive success for both sexes with corresponding changes in shared life-history traits compared to the female biased populations. Divergence between the two treatments is further supported by male biased populations showing increased protection from a natural viral pathogen. Post-copulatory sexual selection was examined in males through the sterile male method. Males evolving under a male biased sex ratio were subject to increased selection on traits involved in sperm competition from rival males' ejaculates, yet sired the same total number of offspring as males evolving under a female biased sex ratio. Sexual selection is a non-mutually exclusive force acting in tandem with sexual conflict, potentially causing increased reproductive success and immunity protection in the male biased populations.

Fecundity selection was examined in a stock population of *P. interpunctella*. Lifetime fecundity was increased without corresponding changes or trade-offs with body size or longevity, two life-history traits known to be important to increased reproductive success in females. Finally, the effects of dietary protein and carbohydrates were examined using the geometric framework. Sexually antagonistic selection over macronutrients was evident, with sexes showing preference for protein and carbohydrate in opposing regions of the nutrient landscape, indicating that intralocus conflict is unlikely to have been resolved in this moth species.



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Will Lerner and Amy Rowley assisted in the laboratory and aided to collect some data for the fecundity selection chapter. Katie Davey and Matt Stephens collected some data for the diet chapter as part of their undergraduate dissertation project.

With these exceptions, I declare that the work contained in this thesis is my own, and has not been submitted for any other degree or award.

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1. General Introduction

1.1 Sexual Selection

1.1.1 Introduction

Natural selection is a key mechanism of evolutionary theory, first proposed by Darwin (1859). As so much variation exists in all organisms, natural selection occurs by targeting specific traits that enhance survival in an individual in relation to the environment in which it is found. At the same time, Darwin (1859) also proposed the theory of sexual selection, as selection for traits that are favourable to an individual as they increase mating success. Sexual selection acts not to increase survival in individuals, but influences traits that aid in securing matings, and thus increase fitness through production of offspring (Darwin, 1859; Andersson, 1994).

Reproductive success is not measured in the same way between the sexes, as males and females typically invest differently in reproduction. Variation in reproductive effort is driven through anisogamy, as males typically produce many small sperm, whilst females tend to produce large, costly eggs (Trivers, 1972). Typically, in order to increase their respective fitness, males attempt to maximise their reproductive success by mating with as many females as possible (Bateman, 1948), in contrast to females who will mate with one or a few 'high quality' males in order to produce as many good quality offspring as she can over her lifetime (Trivers, 1972). Intersexual selection between the sexes occurs, as well as intrasexual selection, as some males/ females have advantages over others in the population for matings. With the evolutionary interests of males and females being so diverse, it is no surprise that

sexual selection can occur at both the pre-copulatory and post-copulatory stage within species (Andersson, 1994).

1.1.2 Pre-Copulatory Sexual Selection

Sexual selection often occurs before males and females begin mating. Both sexes show a diverse range of pre-copulatory mechanisms in order to gain a successful copulation, resulting in viable offspring. Two broad categories of selection occur at the pre-copulatory stage. Intrasexual selection favours traits that are beneficial to the most abundant sex ready to mate, typically males, when in competition with other males. The second is intersexual selection, that favours traits which increase a male's attractiveness to choosey females.

1.1.2.i Intrasexual selection

Males typically compete with each other over the limiting resource, receptive females. This can lead to male-male competition, which sees males in combat for copulations, thereby increasing their own fitness. A number of traits may be selected on in order to gain the advantage in intrasexual competition. Often, large male size is favoured in order to successfully assert dominance in competitions against other males for access to females, or is preferred by females (Andersson, 1994). However, a smaller male body size can sometimes be advantageous, for example in order to avoiding cannibalism and thus ensure mating success in *Nephila edulis*, the golden orb-web spiders (Schneider *et al.*, 2000), or increased agility in the midge *Chironomus plumosus* (Neems *et al.*, 1998).

Direct combat between males can also occur, and in this case weaponry can be advantageous. Weapons are a secondary sexual characteristic that can take many diverse forms (Emlen, 2008). One example is the antlers in red deer, *Cervus elaphus* (Clutton-Brock *et al.*, 1979), where males often engage in aggressive fights to defend their harem. Weapons are not unique to mammals, and a wide range of invertebrates have developed sexually dimorphic weapons such as horns in *Onthophagus*, the dung beetle genus (Emlen *et al.*, 2005), larger head sizes in *Hemideina crassidens*, the wellington tree weta (Kelly, 2004), alongside many others (reviewed in Emlen, 2008). Male armaments are often assumed to be costly to produce, yet recent research in *Trypoxylus dichotomus*, the aptly named rhinoceros beetle, revealed no fitness costs to males in producing their elaborate horns (McCullough and Emlen, 2013).

Mate-guarding is another pre-copulatory strategy frequently used by males to compete against other potential sires. A male may choose to guard a female until she is mature and becomes receptive (Elias *et al.*, 2014). By guarding a female, a male may ensure higher paternity for his sperm investment by delaying female re-mating with rival males (Elias *et al.*, 2014). A further extension of guarding is seen through the use of copulatory plugs, present seen in rodents, bats, marsupials and insects for example. Copulatory plugs are a coagulated protein mass, that are produced by secretions from the male accessory glands, and transferred to the female after mating has occurred by a successful male (Voss, 1979; Uhl *et al.*, 2010). Both mate-guarding and copulatory plugs act as pre-copulatory mechanisms, in order to deter competitor males from mating with the female, but also as post-copulatory mechanism, in order to gain higher paternity of offspring by reducing risk of sperm competition occurring.

1.1.2.ii Intersexual Selection

Whilst males compete with each other to secure a copulation females frequently choose between potential mates. Females may choose males based on direct fitness benefits, or indirect benefits to future offspring (Andersson and Simmons, 2006). Direct fitness benefits to females can include paternal care provided by males, territories, protection from predators, and obtain sufficient amount of sperm to ensure fertilization (Møller and Jennions, 2001). Female fitness is raised by increased fecundity and fertility of eggs. Females may choose males based on traits displayed at courting. However, pre-existing biases in the females' sensory system may be exploited by males during courtship (Fuller *et al.*, 2005), as is the case for coloured fin spots in a number of wild guppy populations (Rodd *et al.*, 2002; Kerino *et al.*, 2002; Sathayon and Couldridge, 2013). As females show a preference for a particular colour, males that display the colour on their tail fin will more likely be chosen as a potential mate.

Females can choose mates based on sexually selected traits, which are passed on to sons in the Fisher process (Fisher, 1930). These heritable attractive traits increase the sons' reproductive success as females preferentially mate with them. Daughters simultaneously inherit the preference for the attractive trait in question. Self-reinforcing selection may be generated through the Fisher process, as there is strong positive selection between male trait and female preference generating a genetic covariance between male trait and female preference. This can lead to traits being expressed in populations despite the cost to males of maintaining them (Kirkpatrick, 1982). Female mate choice can drive the evolution of these attractive, and exaggerated male traits, such as tail feathers in peacocks and widow birds.

Ornaments are secondary sexual traits favoured by females, as they increase a male's attractiveness. Examples of ornaments include the elaborate tails of long-tailed widow birds, *Eupeptis prona* (Andersson, 1982) and the peacock, *Pavo cristatus* (Loyau *et al.*, 2005), elongated eyespan in *Telopsis dalmanni*, stalk-eyed flies (Cotton *et al.*, 2010) and acoustic signalling in field crickets (Bertram and Rook, 2012; Harrison *et al.*, 2013). Ornaments are suggested to allow a male to honestly advertise his condition, as only males in good condition can afford the resources to allocate towards the development of these costly secondary sexual traits, which increases the chances of successful mating and production of viable offspring (Fisher, 1915; Møller and Alatalo, 1999). When males are unsuccessful in combat or courting a female, they may instead try to use sneaky mating tactics as an opportunistic approach. The guppy genus *Poecilia* is a classic example of alternative mating tactics (Pilastro and Bisazza, 1999; Matthews and Magurran, 2000). By gaining copulations through alternative tactics, costs are likely to be minimal to males as they spend less time courting females (Pilastro and Bisazza, 1999).

Mating can be a costly affair for females, due to the energetic demands in producing large ova (Trivers, 1972). Costs may also be incurred before and during mating, such as seen in *Scatophaga stercoraria*, the yellow dung fly, where intense struggles within and between the sexes can ultimately lead to female death (Parker, 1970a). Forced copulations from the male, as seen in monarch butterflies, *Danaus plexippus* for example can lead to fitness reduction for both males and females (Pliske, 1975; Rothschild, 1978). Yet despite this, polyandry, female multiple mating, is a widespread phenomenon, with females seeking copulations, suggesting that the benefits outweigh the costs (Arnqvist and Nilsson, 2000). In resource-based mating systems, males may provide females with territories, and parental care for offspring.

Direct genetic benefits may be provided in absence of material benefits (Jennions and Petrie, 2000). Genetic benefits of polyandry may occur, such as reducing costs of inbreeding depression by increasing genetic variation in offspring, for example in the field cricket *Gryllus bimaculatus* (Tregenza and Wedell, 2002), and the Mediterranean flour moth, *Ephestia kuehniella* (Xu and Wang, 2009). Multiple mating also promotes sperm competition, and may result in production of better quality offspring if the quality of the male is directly related to his sperm competitive ability (Hosken *et al.*, 2003).

Nuptial gifts are resources that males can supply prior to and during mating. These nuptial gifts may vary in quality, from low to high and that can increase the nutritional benefit of mating to the females (reviewed in Gwynne, 2008). Spermatophores can contain nutrients and lead to increased fecundity, such as in some bushcrickets for example (Gwynne, 2001; Ortiz-Jimenez and Cueva del Castillo, 2015), and in *Pieris napi*, the green-veined white butterfly (Wedell, 2006). Females may discriminate between males based on the size of their nuptial gift using phenotypic cues such as wing colour in *Battus philenor*, the Pipevine swallowtail butterfly for example (Rajyaguru *et al.*, 2013). Spermatophores can vary in their function, such as to provide parental care and increase offspring numbers through provision of extra resources (reviewed in Lewis and South, 2012). They can also increase male fertilisation success by enhancing sperm transfer, for example by providing females with a nuptial gift that she feeds on during insemination (Vahed, 1998). Nuptial gifts can represent a significant investment in reproduction by males (Sakaluk *et al.*, 2004). However, as females cannot always assess the nutritional benefits of nuptial gifts until copulation, sexual conflict over the nutritional value of gifts can occur between the sexes (Gwynne, 2008; Gershman *et al.*, 2013).

1.1.3 Post-Copulatory Sexual Selection

Pre-copulatory strategies are not the only driving force in sexual selection. Polyandrous females are abundant throughout the animal kingdom that generates selective forces also after mating. Sexual selection can occur through sperm competition (intrasexual selection) and cryptic female choice (intersexual competition).

1.1.3.i Sperm competition

Post-copulatory sexual selection through sperm competition is a pervasive evolutionary force, capable of altering male reproductive behaviours as well as anatomy over time (Birkhead *et al.*, 2008). Sperm competition occurs when females mate with and store sperm of multiple males that compete to fertilise the ova (Parker, 1970b). Males can maximise their reproductive success through defensive adaptations to protect their ejaculate against a competitor male, or offensive adaptations to overcome a rival males ejaculate when mating with an already inseminated female. Sperm competitive success may be quantified through proportion of eggs sired in first male (P_1 , defensive) or second male roles (P_2 , offensive) (Boorman and Parker 1976). A fair raffle of sperm competition assumes that each male will have a proportionate share of paternity based on the number of sperm provided to the female (Parker, 1990). So a P_1 value of 0.5 indicates complete sperm mixing where males share equal paternity of offspring sired. However, unequal paternity shares are commonplace, and some species show sperm precedence in either the first male (P_1) or more commonly second/ last male roles (P_2). There are many empirical studies showing clear patterns of male sperm precedence (reviewed in Simmons 2001). For example, male *Drosophila*

melanogaster show second male precedence, and their P_2 is correlated with their overall lifetime reproductive success (Fricke *et al.*, 2010).

Risk (likelihood of female remating) and intensity (how many ejaculates competing over eggs) of sperm competition impose selection favouring adaptations that are advantageous in increasing males' paternity shares (Parker, 1970b). As the intensity and risk of sperm competition differs, so may the selection pressures that act on traits associated with P_1 and P_2 (Bernasconi and Keller 2001). In the presence of no other males, models suggest that mating males should provide a smaller amount of ejaculate. Risk models suggest if a female is likely to remate, males should increase ejaculate expenditure in order to increase paternity (under fair raffle system), or to displace a previous males ejaculate (Parker, 1998). This in turn can lead to larger testis evolving in species subject to sperm competition, such as male dung flies (Hosken *et al.*, 2001). As the intensity of sperm competition increases, males competing with more than two ejaculates should decrease their sperm investment per mating due to diminishing returns (Parker *et al.*, 1996, 1997; Wedell *et al.*, 2002).

Males may adjust their reproductive behaviour according to different mating conditions. This can be experimentally examined either by subjecting males with single and multiple female mating treatments (Hosken *et al.*, 2001), or by altering the adult sex ratios (Wigby and Chapman, 2004; Ingleby *et al.*, 2010), thereby creating environments where the risk and intensity of sperm competition is altered. Increased risk and intensity of sperm competition is likely faced in environments where males outnumber females, leading to increased likelihood of female multiple mating. Experimental evolution studies have measured the consequences of increased sperm competition faced by species over time, such as yellow dung flies, *S. stercoraria* (Hosken *et al.*, 2001), *D. melanogaster* (Wigby and Chapman, 2004) and the Indian

meal moth, *P. interpunctella* (Ingleby *et al.*, 2010). Males evolved increased investment in sperm production in response to increased risk and intensity faced through sperm competition.

Strategic sperm allocation can occur. Despite sperm being relatively small, males should maximise their reproductive returns by tailoring sperm numbers and ejaculates (Dewsbury, 1982). Once thought to be relatively cheap to make, recent research reveals that sperm production has associated costs to males (Wedell *et al.*, 2002), even though not as high as the costs to females of producing eggs (Hayward and Gillooly, 2011). Males may be able to assess if a female is a virgin or previously mated. For example, when mating with a non-virgin female, males of the white butterfly (*Pieris rapae*), tailor their ejaculate and donate more sperm due to increased risk of sperm competition (Wedell and Cook, 1999). Males may also adjust the size of their ejaculates according to their alternative reproductive strategies. Dung beetles, *Onthophagus*, either compete for females or sneak copulations. Males who sneak copulations are found to have increased ejaculate size, larger testes, and longer sperm due to their increased risk of sperm competition, compared to males who guard their females (Simmons *et al.*, 1999).

Sperm competition may target other ejaculate traits to aid in fertilisation success rather than just increasing relative sperm numbers. Sperm size is predicted to be small and populous rather than larger in size (Parker, 1982). Yet, some species show an increase in sperm length under higher risk of sperm competition, such as across moth species (Morrow and Gage, 2000). Longevity and motility of sperm may also be affected by sperm competition (Snook, 2005). Sperm polymorphism is also proposed to be a result of males evolving under different pressures of sperm competition (Simmons, 2001). Different morphs of sperm may be present in an ejaculate, yet only

one morph may fertilise eggs. For example, *Drosophila pseudoobscura* produce both long and short sperm, yet only the long sperm are used in fertilisation of eggs (Snook, 1997). Lepidoptera also exhibit sperm polymorphism with males producing both normal fertile sperm (eupyrene), and non-fertile sperm (apyrene) that do not fertilise eggs (Silberglied *et al.*, 1984). Non-fertile sperm are suggested to delay female remating through filling up the spermatheca (Cook and Wedell, 1999; Morrow and Gage, 2000).

In order to reduce the risk of sperm competition, males can adopt defensive mechanisms, such as post-copulatory mate-guarding, prolonged copulation durations, and decreasing female receptivity by transferring accessory gland proteins at mating that affect female reproductive physiology, such as observed in *Drosophila melanogaster* (Wigby and Chapman, 2005). Copulatory plugs have been observed to increase in size in rodents in response to sperm competition (Ramm *et al.*, 2005).

1.1.3.ii Cryptic Female Choice

Post-copulatory mechanisms are not just driven through male sperm competition, and it is now widely recognised that females can bias the paternity of offspring after mating, termed cryptic female choice (CFC) (Eberhard, 1996). It is less obvious how females can favour sperm from one male over the other, and is harder to test for empirically than sperm competition (Birkhead, 1998; Pitnick and Brown, 2000). A wide variety of mechanism are suggested as ways for CFC to operate such as lack of ovulation, lack of sperm transport to storage organs, failing to modify insemination ducts, and not maximally investing in the mates offspring to name but a few (reviewed in Eberhard, 1996). Whilst these mechanisms are difficult to observe, research suggests that CFC is a powerful evolutionary force and that females are not passive in

post-copulatory selection mechanisms (Eberhard, 1996). CFC may drive pre-copulatory selection for attractive male traits, as observed in *P. reticulata* for female preference for orange tail spots (Pilastro *et al.*, 2004).

Female choice in biasing paternity can be as simple as mating with another male, especially in a species such as *D. melanogaster* who have second male precedence (Fricke *et al.*, 2005). A female may also choose to reduce or terminate copulation, which minimises the males' paternity share of eggs. Copulatory plugs provided by males to secure their sperm investment can be removed by females. Sperm dumping by females can also occur, in order to favour a particular male based on traits such as copulatory courtship displayed by males in the spider *Physocyclus globosus* for example, that can lead to females favouring sperm from the more attractive male (Peretti and Eberhard, 2010). In feral fowl *Gallus gallus domesticus* for example, males often sexually coerce females into mating, and females eject ejaculates from subordinate males so that dominant male phenotypes were favoured (Pizzari and Birkhead, 2000).

Post-copulatory mechanisms are known to exert sperm precedence in order to reduce the costs of inbreeding, such as in the house mouse *Mus domesticus* (Firman and Simmons, 2008) and field cricket *Teleogryllus oceanicus* for example (Tuni *et al.*, 2013). However, the exact mechanisms remain unclear. In *Argiope lobate*, the orb-web spider, inbreeding avoidance occurs through cryptic female choice with females storing more sperm in the spermatheca from unrelated mates over siblings (Welke and Schneider, 2009). Ovarian fluid in externally fertilising organisms can mediate CFC for unrelated males, as observed in the guppy, *Poecilia reticulata* for example, by slowing down swimming speeds of related males' sperm (Gasparini and Pilastro, 2011). Ovarian fluid is also shown to increase sperm transfer and motility of

conspecifics over heterospecific sperm in experimental manipulation of trout and salmon eggs (Yeates *et al.*, 2013).

Female multiple mating may also be driven through genetic compatibility between the two sexes (reviewed in Tregenza and Wedell, 2000). If sperm from some males are more genetically compatible and increase viability of eggs, or if females deem males to be better sires for their offspring, then genetic compatibility selection may occur post-copulation. Incompatibility may be driven through avoidance of inbreeding depression (Pusey and Wolf, 1996), as well as the presence of selfish genetic elements (Zeh and Zeh, 1996, and Zeh and Zeh, 1997, Price *et al.*, 2008).

1.2 Sexual Conflict

1.2.1 Introduction

As discussed above, the reproductive interests of males and females are vastly different, largely driven through anisogamy. Sexual selection acts at both the pre- and post-copulatory stage in order to increase successful copulations and viable offspring production. However, as the sexes contrast in what they require in order to increase their own relative fitness, this can create sexual conflict. Sexual conflict is just as ubiquitous as sexual selection, and there are two main types of conflict (Rice and Chippindale, 2001; Arnqvist and Rowe, 2013) that are discussed below.

1.2.2 Interlocus Sexual Conflict

The first type of conflict is termed interlocus sexual conflict (IRSC) and it arises due to interaction of alleles at different loci between the sexes (Chapman *et al.*, 2003).

Although males and females reproduce together, they often have differing optimal outcomes from these interactions, leading to IRSC. Conflicts are likely to occur over fertilisation, parental effort and clutch size (Arnqvist and Rowe, 2013). Mating frequencies between the sexes are also likely to be a source of conflict, as observed in the water strider *Gerris incognitus* (Arnqvist and Rowe, 1995). Male *Drosophila melanogaster* provide females with ejaculate containing seminal fluid proteins as well as sperm. Seminal fluid proteins have a wide variety of functions of benefit to males by altering females' reproductive behaviour and reducing the likelihood of female remating and increasing oviposition rates (Chapman and Davies, 2004). However, these proteins carry a cost to females, and can cause a reduction in lifespan (Wigby and Chapman, 2005). *D. melanogaster* demonstrate a clear conflict of males trying to increase their own fitness optimums, at the expense of lifespan in females.

IRSC can cause the sexes to evolve counter-adaptations arising from reproductive interactions, potentially leading to co-evolutionary arms races between the sexes (Parker, 1979). Marked phenotypic traits can evolve in response to these conflicts, such as spiked genitalia, amongst others (reviewed in Arnqvist and Rowe, 2013). For example, both sexes in a number of water striders species are seen to co-evolve armaments to either better grasp the females by males, or to allow females to reject males in their pre-copulatory struggles (Arnqvist and Rowe, 2002; Perry and Rowe, 2012). Sexual conflict faced by the sexes in high and low density populations can lead to divergence of populations in mating rates as observed in the dung fly *Sepsis cynipsea* (Martin and Hosken, 2003). Speciation as a result of sexual conflict has been discussed theoretically (Gavrilets, 2014).

1.2.3 Intralocus Sexual Conflict

The second type of conflict faced by the sexes is intralocus sexual conflict (IASC). Intralocus conflict arises when the same allele is expressed in both the sexes but has contrasting fitness effects (Bonduriansky and Chenoweth, 2009; Arnqvist and Rowe, 2013). Termed sexually antagonistic alleles, conflict arises due to many shared traits having a common genetic basis in the sexes, but selected for in opposite directions (Price and Hosken, 2007). If traits are selected for in opposing directions, we may see one sex being driven away from its phenotypic optimum while the other sex shows increased fitness (Chippindale *et al.*, 2001; Van Doorn, 2009).

IASC can be quantified through the intersexual genetic correlation between the sexes (r_{mf}) for traits and fitness, which measures the additive effects of alleles and their similarity in the sexes (Bonduriansky and Chenoweth, 2009). When $r_{mf} = 1$, there is a strong and positive genetic correlation for the trait in the sexes. However, when $r_{mf} < 0$, it indicates that sexes oppose in their optimums for the trait in question, causing a negative intersexual correlation. Bonduriansky and Chenoweth (2009) identify and discuss four phases of IASC, *i*) before IASC, *ii*) acute IASC, *iii*) attenuated IASC and *iv*) resolved IASC. Phases *i* and *iv* are representative of stabilising selection acting on fitness and the traits in question. This results in the intersexual additive genetic correlation, r_{mf} , being equal to 1. . Phase *ii* and *iii* are more akin to $r_{mf} < 0$, when trait values and fitness are opposing between the sexes. Opposing trait values is when IASC is thought to be at its strongest between the sexes. A genomic tug-of-war can occur, and as selection favours a trait in one sex, a correlated response may be generated in the other leading to reductions in fitness. A gender load may be imposed by intralocus sexual conflict, leading to the population as a whole showing reduced fitness (Arnqvist and Tuda, 2010).

Although not as easily quantified in populations as interlocus conflict, a seminal paper demonstrating the negative genetic correlation for fitness between the sexes in *Drosophila melanogaster* causing researchers to switch focus (Chippindale *et al.*, 2001). Empirical demonstration of IASC has since occurred in both wild and laboratory adapted populations. Foerster *et al.* (2007) showed that in a wild population of red deer, high fitness males produced daughters of low fitness. Conflict has been shown to occur over immune defence between the sexes in side-blotched lizards, *Uta stansburiana* (Svensson *et al.*, 2009), as well as over diet choices in the field cricket, *Telogyllus commodus* (Maklakov *et al.*, 2008), with males and females being constrained in their optimal eating patterns. Life-history traits in the seed beetle, *Callosobruchus machulatus* are shown to be subject to sexually antagonistic selection (Berger *et al.*, 2014). Human hip width is another suggested example, with the allele for smaller hips being favoured in men, which are optimised for walking. However, if this allele is expressed in females it can lead to potentially harmful consequences during childbirth, causing larger hips being favoured in females in order to minimise danger to the mother (Rice and Chippindale, 2001).

1.2.4 Resolving Intralocus Sexual Conflict

Due to many traits being shared between the sexes, it becomes increasingly clear that IASC is a prevalent force, liable to shape a species evolutionary trajectory (Bonduriansky and Chenoweth, 2009). IASC is suggested to be a transient force, able to alter genetic architecture and ultimately lead to conflict resolution (Cox and Calsbeek, 2009). A number of ways for this to happen have been suggested (reviewed in Pennell and Morrow, 2013), that are discussed below.

1.2.4.i Sex-biased Gene Expression

Theory suggests that the sexes may evolve sex-specific gene expression patterns, which would help to overcome the constraints imposed by intralocus sexual conflict. This could occur in a number of ways, allowing sexes to reach optimal sexual dimorphism for the traits in question. One such method proposed to achieve sex-biased gene expression is via hormonal cascades. Levels of testosterone in mammals (Hau, 2007) and juvenile hormone in insects (Emlen *et al.*, 2006) not only differ between the sexes, but can lead to secondary trait expression. Sex-specific gene expression may be driven through intra-cellular signalling caused by varying hormone levels. This in turn may favour gene expression (or repression) in one sex, leading to dimorphic gene expression in the sexes that may resolve IASC.

Gene duplication is another mechanism proposed to overcome the effect of sexually antagonistic alleles (Gallach and Betrán, 2011). By duplication and translocation of genes, they are free to evolve sex-specific functions in both sexes without the constraints imposed by selection favouring trait values in either males or females (Ellegren and Parsch, 2007; Gallach and Betrán, 2011). It was originally proposed that genes should be translocated to the sex chromosomes in order for sex-biased gene expression to occur (Charlesworth and Charlesworth, 1980; Bachtrog, 2006). However, complications will arise if the genes being relocated are required for functions in the opposite sex. Hosken (2011) makes the argument that as so many traits are genetically correlated between the sexes, sex-biased expression may still be constrained by the underlying genetic architecture.

1.2.4.ii Sexual Dimorphism

As the sexes typically have contrasting fitness optima, it has been suggested that achieving sexual dimorphism should represent conflict resolution (Lande, 1980). Genetic architecture for a trait may be altered, allowing favourable trait evolution to occur in both sexes independently of each other, leading to sexual dimorphism. Once sexual dimorphism has been reached, it may remain fairly robust to opposing selection pressures. Artificial selection in opposing directions was applied to body size in the dimorphic flour beetle, *Tribolium castaneum* (Tigreros and Lewis, 2011). After a number of generations it was observed that no significant changes in body size measures had occurred between the sexes, causing sexual size dimorphism to remain the same. IASC may be resolved as sexes reach their optima for specific traits that relate to fitness.

However, recent work has shown that species can evolve sexually dimorphic traits, and still be constrained by intralocus sexual conflict (Cox and Calsbeek, 2009). Selection for increased mandible size, a dimorphic male trait in *Gnathocerus cornutus*, the broad-horned flour beetle, was found to lower fitness in females from the same populations, indicating a negative intersexual genetic correlation that is the signature of IASC (Harano *et al.*, 2010). This was due to mandible size being genetically correlated with abdomen size, a trait important to female fecundity. Lewis *et al.* (2011) also find evidence for strong IASC between the sexes in *Plodia interpunctella* for shared life-history traits, despite the sexes being sexually dimorphic.

IASC resolution depends upon genetic architecture evolution, as selection on specific traits may cause a shift in IASC as correlated changes in a secondary trait may

be detrimental to one/ both sexes. Traits are rarely genetically independent, and genetic architecture may inhibit conflict resolution. Importantly, sexual dimorphism itself may not be indicative of conflict resolution (Bonduriansky and Chenoweth, 2009).

1.2.4.iii Sex-Ratio Adjustment of Offspring

A further way for individuals to overcome IASC is through sex-ratio adjustment (SRA) of offspring. Rather than 'wait' for genetic architecture evolution and sex-biased gene expression to occur, individuals can adjust their ratio of sons/ daughters in order to mitigate the fitness costs imposed by sexually antagonistic conflict.

A study of wild side-blotched lizards, *Uta stansburiana*, found that depending on the phenotype of the male, females can choose sperm in order to select the sex of the offspring (Calsbeek and Sinervo, 2004). If mated with a large male, the female would produce more sons, due to positive correlation between size and fitness in males (and negative correlation for females). As a corollary, small male mates would result in females producing more daughters. A number of other studies have observed SRA in offspring as a way to mask the harmful effects of IASC in the sexes, including brown anole lizards, *Anolis sagrei* (Cox and Calsbeek, 2010), and broad-horned flour beetles, *Gnathocerus cornutus* (Katsuki *et al.*, 2012). Although fitness is assumed to derive from the male's phenotype, broad-horned beetles were found to bias their offspring based on the female's fitness (Katsuki *et al.*, 2012). High fitness females produce more daughters, whereas sons would be produced by low fitness females. Through biased offspring sex ratios, both sexes benefit from higher fitness than what may have been expected under constraints of IASC.

Whilst the exact mechanisms for adjusting offspring sex ratio remain unclear, a simple model proposed by Blackburn *et al.* (2010) reveals that SRA can occur in the presence of IASC, and offers relief from sexual antagonism. Only one gene was examined in the model, but Blackburn *et al.* (2010) argue that if the fitness benefits are greater than the costs imposed by IASC, then SRA should evolve in the presence of many sexually antagonistic genes.

1.3 Reproductive Success

1.3.1 Introduction

Selection shapes strategies that maximise individual lifetime fitness, with individual variation in fitness reflected in phenotypic differences (Bonduriansky *et al.*, 2008). Resources must be acquired and assigned toward traits that increase current and future reproduction such as fecundity and mating success, and often traded-off against survival (Houslay *et al.*, 2015; Pincheira-Donoso and Hunt, 2015). The sexes gain fitness benefits via different reproductive routes, as a consequence of anisogamy (Schärer *et al.*, 2012). Females are typically selected to produce large eggs that carry an increased energetic cost, compared to the smaller sperm in males, (Trivers, 1972; Hayward and Gillooly, 2011). Measures of reproductive output are different between the sexes, as females are limited by the number of eggs they can produce (fecundity), whereas males typically rely on higher mating success in order to fertilize many eggs (Bateman, 1948; Trivers, 1972).

1.3.2 Males

In order to increase reproductive success, males rely on higher mating success in order to fertilise as many eggs for viable offspring (Bateman, 1948). A male may gain copulations through successful courting of females, displaying ornaments to make them more attractive or winning in combat with other males, for example in pre-copulatory sexual selection (see section 1.1.2). As females mate multiply, selection on male traits to confer fitness advantages does not just end at the pre-copulatory stage, and males are often selected to increase traits post-copulation, by engaging in sperm competition (see section 1.1.3.i).

Spermatozoa are selected for a number of morphological traits to increase chances of fertilising eggs under sperm competition, such as mobility, longevity, number and size (reviewed in Birkhead *et al.*, 2009). However, fertile sperm may only make up a small proportion of entire ejaculate, as in the example of *Pieris rapa*, the small white butterfly in which fertile eupyrene sperm are only around 10% of the ejaculate transferred to females (Cook and Wedell, 1996). Ejaculates consist of a whole suite of other components, including water, macromolecules and seminal proteins that in turn may aid fertilisation success of males in post-copulatory selection (Perry *et al.*, 2013). Male accessory gland proteins secreted in seminal fluid of *Drosophila melanogaster* for example have been studied in-depth, and are required for sperm transfer and sperm competition, as well as increasing egg production and decreasing female receptivity to re-mating (Chapman and Davies, 2004; Ram *et al.*, 2005).

Male ejaculate production may be promoted or constrained by other factors. Larger testes may be selected for in order to increase sperm production and/ or aid in multiple mating rates in males, by avoiding sperm depletion (reviewed in Vahed and Parker, 2012). Male condition plays an important role in male mating success, as males

who are in better condition can donate more resources towards sperm and ejaculate production. For example, in the sex role-reversed honeylocust beetles, *Megabruchidius dorsalis*, males who were supplemented with extra food as adults mated more frequently, transferred larger ejaculates, sired more offspring and outlived males who were not food supplemented (Fritzsche and Arnqvist, 2015). Similarly, nutritional stress faced by male *Plodia interpunctella*, the Indian meal moth during development can lead to reduced sperm numbers (Gage and Cook, 1994). This suggests that diet, and ultimate condition of males can lead to increased or decreased reproductive success.

1.3.3 Females

Fecundity selection increases female fitness through selection on traits that confer a fecundity advantage (Pincheira-Donoso and Hunt, 2015). Darwin (1874) first proposed the hypothesis of fecundity advantage to explain female-biased sexual size dimorphism (SSD), as larger body size in females may allow for more offspring to be produced. It was thought that strong selection for increased fecundity drives correlated changes in body size, generating the evolution of female-biased SSD. Female-biased SSD is a pattern commonly found in a majority of invertebrates, as well as some fish and reptile species (Fairbairn, 1997). Research incorporating fecundity theory has now shifted focus to include traits that may confer high fecundity, such as large abdomen size, as for example in the waterstrider *Aquaris remigis* (Preziosi *et al.*, 1996), and tail length in males of the sex-role reversed *Sygnathus* pipefish, which increases area the available to brood eggs (Winkler *et al.*, 2012). Whilst body size positively correlates with increased fecundity in insects, there are selection pressures

in place that inhibits them from evolving to become even larger (Blanckenhorn, 2000). Although female-biased SSD is common, previous research in lizard populations for example suggests that the strength of fecundity selection alone is not responsible for the female-biased SSD patterns (Cox *et al.*, 2003; Pincheira-Donoso and Tregenza, 2011). Sexual selection for small male body size could also drive patterns of asymmetric sexual size dimorphisms, by reducing risk of cannibalism by females in several species of spiders for example (Elgar and Fahey, 1996; Fromhage and Schneider, 2005).

Female reproductive success may be promoted or constrained by other extraneous factors, not just via selection on traits that increase fecundity. Environmental conditions, such as decreasing temperatures, are known to adversely affect fecundity through limiting egg maturation rates and oviposition opportunities as in the speckled wood butterfly *Pararge aegeria*, for example (Berger *et al.*, 2008). Time may be a limiting resource to females, as temperature conditions may vary throughout the day or season, limiting opportunities for egg-laying (Berger *et al.*, 2012). Nutritional resources available to adults can show a marked effect on female fecundity, with diets rich in protein leading to increased reproductive output in decorated crickets, *Gryllodes sigillatus* (Archer *et al.*, 2012; Houslay *et al.*, 2015). However, in holometabolous species such as the tropical butterfly *Bicyclus anynana* for example, larvae that are subjected to food stress during development have reduced fecundity and correlated smaller body size as adults in comparison to larvae that were fed *ad libitum*, as they are unable to compensate larval nutrients through adult feeding (Bauerfeind and Fischer, 2005). For certain species, females are able to supplement their diet by receiving nuptial gifts from males at mating (Lewis and South, 2012). Spermatophores can lead to increased fecundity in females, such as in some bush

crickets for example (Brown, 1997; Gwynne, 2001; Ortiz-Jimenez and Cueva del Castillo, 2015), and in *Pieris napi*, green-veined white butterflies (Wedell, 2006). Female multiple mating is also shown to increase reproductive output in some species, which can outweigh potential costs of mating (Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000). Direct benefits of female multiple mating can include increased fecundity and fertility of eggs, such as in the tomato leafminer moth, *Tuta absoluta* (Lee *et al.*, 2014), as well as genetic benefits of polyandry, such as reducing costs of inbreeding depression by biasing paternity against related males, for example in the field cricket *Gryllus bimaculatus* (Tregenza and Wedell, 2002), and the Mediterranean flour moth, *Ephestia kuehniella* (Xu and Wang, 2009).

Selective forces can vary between the sexes due to differing reproductive strategies. The underlying genetic architecture may facilitate traits that confer a fecundity increase. In female heterogametic species, such as Lepidopterans and birds, it is the female that have two different sex chromosomes i.e. ZW versus ZZ males, compared to male heterogametic species such as humans (XY males versus XX females) (Ellegren, 2011). The genetic architecture is especially important to female heterogametic species, as W-linked genes (only present in females) are thought to play an important role in female fecundity and fertility selection (Bachtrog *et al.*, 2011). A recent study examined the expression of genes on the W chromosome in domestic chickens in comparison to their distant ancestor, the red jungle fowl (Moghadam *et al.*, 2012). It was found that chicken breeds that are under strong female-specific selection i.e. have been bred for increased egg laying rates, show greater expression of female-biased genes on the W chromosome (Moghadam *et al.*, 2012).

1.4 Trade-Offs

1.4.1 Introduction

Whilst sexual selection and sexual conflict favour can drive and favour trait expression in one sex to gain fitness at the expense of the other, trade-offs within individuals may be generated. Resources are finite within individuals (Sheldon and Verhurst, 1996), and as one trait increases its energetic demands, this can occur at the cost of another. Trade-offs within vertebrates and invertebrates over immune response, life-history traits, somatic maintenance and survival are ubiquitous.

1.4.2 Immunity

According to life-history theory, there may be flexibility in the use of resources (energy) to mount an immune response. As organisms have finite amount of resources that are also invested in reproduction, there will be limitations to the immune response an individual can generate (Sheldon and Verhurst, 1996). Trade-offs between reproduction and immunity are common in both vertebrates (reviewed in Martin *et al.*, 2008) and insects (Rolff and Siva-Jothy, 2002; reviewed in Lawniczak *et al.*, 2007). For example, reproductive success is shown to decrease in resistance to varying pathogens in several species of mosquitoes (Ferdig *et al.*, 1993; Ahmed *et al.*, 2002), as well as the Texan field cricket, *Gryllus texensis* (Stahlschmidt *et al.*, 2013).

Immune response may be condition-dependant, with animals in better condition having extra resources to mount a more effective immune response than those exposed to unfavourable conditions. Immunity will be affected by the diet available to an organism leading up to a pathogen attack (e.g. Moret and Schmid-Hempel, 2000; Fellous and Lazzaro, 2010). For example when given the choice of a

variety of diets, the caterpillar *Spodoptera littoralis* was found to select and change diet based on macronutrient composition depending on whether they were infected with a pathogen or not (Cotter *et al.*, 2011). This suggests that diets that are optimal for fighting disease are not suitable for growth and somatic maintenance, leading to trade-offs in an individual. Varying other factors can also generate a positive or negative effect on immunity within individuals, such as environmental temperatures (Frid and Myers, 2002; Karl *et al.*, 2011) and population density (Barnes and Siva-Jothy, 2000; Wilson *et al.*, 2002).

Sexual selection and conflict may drive trade-offs, leading to immunity being invested in by one sex over the other depending on reproductive strategies. It is suggested that as males typically adopt a “live fast, die young” approach (Trivers, 1972; Rolff, 2002), with immunity down-regulated, in favour of resources invested towards mating. Age-dependant trade-offs between reproduction and immunity is present in male cotton bollworm, *Helicovera armigera* for example, but not in females (McNamara *et al.*, 2013a). Hamilton and Zuk (1982) proposed the idea that sexual selection may be mediated by parasites, as only males who have a good immune function can afford to invest in their secondary sexual characteristic, ornaments. This leads to females selecting males based on their attractiveness, ensuring good genes are transferred as well as avoiding disease transmission (Møller *et al.*, 1999).

1.4.3 Life-History Traits

Life-history theory is also based on the premise that resources are limited within individuals, and allocating resources towards one trait can lead to a reduction in another trait (reviewed in Zera and Harshman, 2001). *Drosophila melanogaster* are a

useful study organism of life-history trade-offs, as much work has shown that if selected for shorter development times, adult size and fecundity will be reduced (Nunney, 1996; Chippindale *et al.*, 1997). *D. melanogaster* selected for longer lifespan show reductions in early fecundity (Partidge *et al.*, 1999), yet recent research now indicates that these two traits may be uncoupled as trade-offs are not always generated (Khazaeli and Curtsinger, 2013; Wit *et al.*, 2013). Frequently in a lot of insects, achieving a larger body size requires longer development times, which in turn negatively impacts on survival rates as individuals are exposed to more diseases as well as competing over food resources (Blanckenhorn, 2000).

Life-history trade-offs may be mediated through condition-dependence. Food availability or shortages whilst developing as larvae can impact on adult body size and development times as shown in the American rubyspot damselfly, *Hetaerina Americana* for example (Jiménez-Cortés *et al.*, 2012). Larval diet can produce trade-offs across generations, as offspring sired from parents raised in poor or rich diets can show trade-offs with fecundity and life-history traits (Valtonen *et al.*, 2012). Dietary restriction is widely recognised as method for increasing lifespan, yet reduces female fecundity (Adler *et al.*, 2013). As well as being context-dependant, diet influences are shown to be sex-specific (Adler *et al.*, 2013).

1.4.4 Survival

Often when resources are invested in reproduction, or size, it is traded-off at the expense of lifespan. Survival costs are commonplace in both sexes in *Drosophila* when reproductive effort is increased (Flatt, 2011). In many insects resources are allocated towards the energetically high demands of egg production at the expense of somatic maintenance required for longer life (Chapman *et al.*, 1998; Partridge *et al.*, 2005). For

example, female *P. interpunctella* moths frequently exhibit shorter lifespans when reproductive output is higher, generating a trade-off between fecundity and longevity (Lewis *et al.*, 2011).

Body size can affect survival rates, as investment into a larger phenotype can cause senescence to occur rapidly, as is the case for domestic dogs, *Canis lupus familiaris* (Kraus *et al.*, 2013). Investing energy into a large body size may not just be detrimental an individual, but also to their offspring. For example, a larger body size of adult Mediterranean flour moths, *Ephestia kuehniella* leads to reduced offspring survival rates (Xu and Wang, 2013).

Lifespan extension may be achieved through dietary restriction. It has been shown in *D. melanogaster* that longer lifespans can be obtained through nutrient reduction, without starvation/ malnutrition (Metaxakis and Partridge, 2013). Yet diet affects both lifespan and reproduction, and when given a choice, the field cricket *Teleogryllus commodus* for example, will favour diets that are more beneficial to sex-specific reproduction over lifespan extension (Maklakov *et al.*, 2008). However, the sexes may be constrained in their optimal eating patterns, as diets that are good for males may be bad for females, and *vice versa* (Maklakov *et al.*, 2008). So not only are lifespan and survival traded-off within the sexes, but males and females may inhibit each other from obtaining the correct nutrients to increase their relative fitness optima if diet traits are genetically correlated between the sexes .

1.5 *Plodia interpunctella*

1.5.1 Introduction

The Indian meal moth, *Plodia interpunctella*, is the model species used throughout this thesis. Part of the Lepidopteran family Pyralidae, they have been extensively studied, and are particularly useful in evolutionary studies. *P. interpunctella* are a global pest species of stored grains. Laboratory conditions can replicate the conditions that *P. interpunctella* is found in the wild, allowing measures of life-history traits and fitness to be comparable to natural conditions.

1.5.1 Females

The Lepidoptera is divided into two main groups based on female genital morphology (Dugdale, 1974). The monotrystian females have only a single genital opening used for copulation and oviposition, and are the more primitive group. The ditrystian group make up the majority of the Lepidopteran order, and have paired genital openings in order to separate copulations from egg laying (Dugdale, 1974), and *P. interpunctella* are a species within the ditrystian group. The reproductive anatomy of a ditrystian female is illustrated below (Figure 1.1). Throughout most of their adult life, female *P. interpunctella* produce pheromones in order to attract mates (Brady and Smithwick, 1968; Grant *et al.*, 1975). During copulation, a male transfers a spermatophore containing both fertile, eupyrene and non-fertile apyrene sperm, into the female bursa. After a few hours, sperm migrate or are transported to the female sperm storage organ, the spermatheca, until required for fertilisation of eggs. Once sperm has left the spermatophore, the remnants remain inside the bursa throughout

the females' life, allowing the number of times a female has mated to be determined (Drummond, 1984).

Female *P. interpunctella* are a mildly polyandrous species, with females mating around 1.79 times on average across their lifespan (Cook, 1999). There are no direct fecundity benefits from multiple mating in *P. interpunctella* and one mating is enough to ensure complete fertilization of eggs produced by females (Cook, 1999). Yet, female *P. interpunctella* has shown response to manipulation of adult sex ratio through experimental evolution, with females mating 2.3 times in a male-biased regime (3 times as many males), in contrast to only 0.97 times in a female-biased (3 times as many females) selection regime over a period of 3 days on average (Ingleby *et al.*, 2010). Altering mating rates and frequencies of females through experimental evolution generates variation in sperm competition, in turn increasing selection pressures on males both at the pre- and post-copulatory stages under a male-biased adult sex ratio.

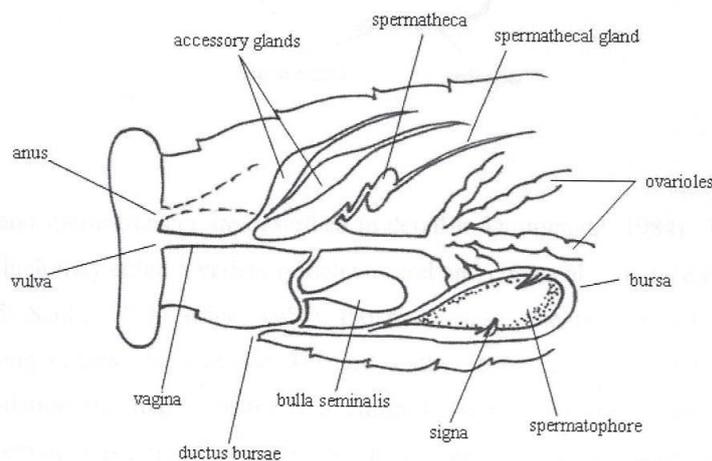


Figure 1.1: Internal reproductive system of female ditrypsian Lepidoptera (image from Lewis, 2005).

1.5.3 Males

Male *Plodia interpunctella* actively seek out and court females, using a variety of chemical and behavioural stimuli (Grant and Brady, 1975; Grant *et al.*, 1975). During courtship, males release a sex pheromone from the base of their forewings, which encourages females to accept copulation. Males then clasp the female abdomen with paired clasping organs, the valvulae. Once copulation commences with a female, the male constructs a spermatophore within the females bursa, with sperm transfer occurring just before copulation ceases (Drummond, 1984), and the male releasing the female. The reproductive anatomy of the male Lepidoptera is illustrated below (Figure 1.2). Male testes are fused together, and are pigmented, rendering them visible through the dorsal body cavity wall in developing larvae from 4th instar onwards (Ingleby *et al.*, 2010). This allows larvae to be sexed before becoming sexually mature adults, ensuring virginity upon eclosion of the moths.

As discussed above for females, males too respond to experimental evolution, as a response to sperm competition generated through female multiple mating. Males mate on average only 0.77 times in the male-biased regime, in contrast to 2.9 times in the female-biased regime over a period of 3 days (Ingleby *et al.*, 2010).

Spermatophores can be a costly investment for males, with successive spermatophores containing fewer sperm in response to nutritional stress (Gage and Cook, 1994). Strategic sperm allocation has been documented in *P. interpunctella*, with males ejaculating greater numbers of fertile sperm in response to the presence of sperm already stored in multiple mated females (Cook and Gage, 1995). This is further corroborated through experimental evolution with males showing an evolved response to increased sperm competition by producing and donating larger ejaculates containing more fertile sperm (Ingleby *et al.*, 2010).

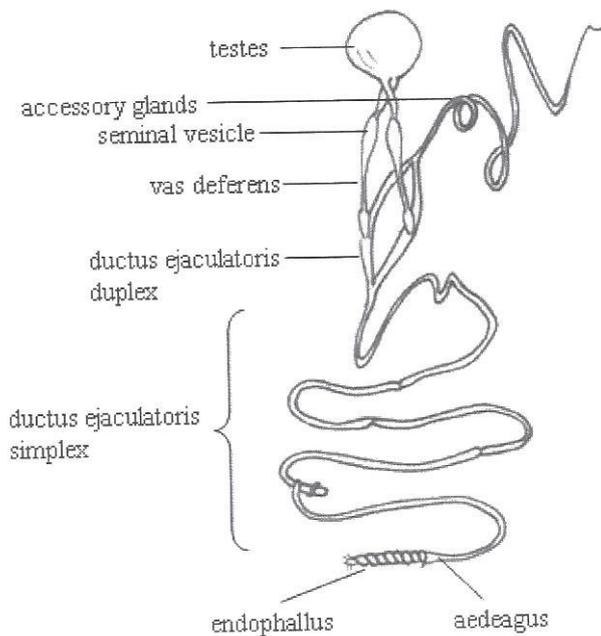


Figure 1.2: Internal reproductive system of male Lepidoptera (image from Lewis, 2005).

A unique feature of Lepidopteran males is the evolution of sperm polymorphism. Males produce two types of sperm: non-fertile “apyrene” sperm, and fertile “eupyrene” sperm (Friedländer, 1997). Non-fertile sperm are distinguished through lack of a nucleus, leaving them unable to fertilise ova, as well as being shorter and thinner than eupyrene sperm (Friedländer and Wahrman, 1970). Despite not being able to fertilise eggs, apyrene sperm travel faster to the spermatheca than fertile sperm (Watanabe *et al.*, 2000), and make up ~ 95% of an ejaculate (Cook and Wedell, 1996). This suggests that apyrene sperm have an adaptive value to Lepidopterans. They are proposed to be cheaper to produce than fertile sperm and to fill the spermatheca in order to delay remating by ‘tricking’ the female to think she has many stored fertile sperm. For example, in *Pieris napi*, the green-veined white butterfly, the

amount of apyrene sperm present in the spermatheca is related to female receptivity, with larger numbers stored being associated with delaying mating (Cook and Wedell, 1999). By delaying female remating, males are able to reduce the potential for sperm competition. This may lead to sexually antagonistic coevolution between the sexes, with males producing increasingly more non-fertile sperm, and females resisting being 'tricked', requiring larger number of apyrene sperm to be transferred in order to suppress female receptivity.

1.5.3 Intralocus Conflict and *Plodia interpunctella*

Recent research in stock populations of *Plodia interpunctella* demonstrates multivariate selection acting on three shared life-history traits (development time, longevity and body size) that are correlated with fitness (Lewis *et al.*, 2011). Selection on these shared life-history traits is opposing in the sexes, where a trait combination that favours females being detrimental to males, and *vice versa*. This antagonistic selection is the signature of intralocus conflict (Bonduriansky and Chenoweth, 2009). Life-history traits are likely to be subject to high levels of conflict, given their strong relationship to fitness, the fact they are shared between the sexes, and having differing optima in the sexes (Wedell *et al.*, 2006). In male *P. interpunctella*, high fitness was optimised by rapid development time, and a longer lifespan. In contrast, female fitness was optimised by a longer development time, shorter lifespan and a larger body size (Lewis *et al.*, 2011). It has been suggested that one way to overcome sexual conflict is through evolution of sexual dimorphism (Van Doorn, 2009). Yet despite sexual dimorphism in these life-history traits intralocus conflict remains strong and prevalent in *P. interpunctella* (Lewis *et al.*, 2011).

1.6 Outline and Aims of Thesis

Like most animals, *Plodia interpunctella* males and females maximise their reproductive potential in a variety of ways. This can lead to conflict between the sexes over reproduction. The main aim of this thesis is to investigate how the sexes respond to differing selection pressures and sexual conflict, and how these in turn affect their overall reproductive potential.

Life-history traits have a common genetic basis in the sexes and are correlated with fitness, yet often subjected to contrasting patterns of selection in the sexes. This can, and does lead to intralocus sexual conflict. By creating reproductive environments that favour one sex over the other through experimental evolution, chapter 3 aims to investigate how life-history traits may diverge as a response to sexual conflict faced by moths when mating as adults under different sex-biased regimes. The impact of altering adult mating ratios is also examined on fitness within and between the sexes under male- and female-biased regimes.

Post-copulatory sexual selection can act to increase a male's reproductive success through traits that confer an advantage when engaging in sperm competition. Experimental evolution alters the risk and intensity of sperm competition faced by males, as females mate multiply. Males evolving under male- or female-biased regimes are found to alter their sperm allocation patterns. Chapter 4 investigates whether divergence in ejaculate traits increase sperm defence (P_1) or sperm offence (P_2) ability, and how this relates to male reproductive success overall.

Immunity is costly for individuals to maintain, and is often traded-off against future reproductive episodes. Resources are finite within individuals, and animals in better condition are better able to mount an effective immune response. Experimental

evolution lines of *P. interpunctella* have diverged in titres of phenoloxidase, a key component of the insect immune system. Chapter 5 investigates the effect a species-specific viral pathogen has in the diverged experimental evolution lines of *P. interpunctella*.

Selection shapes strategies that maximise individual lifetime fitness, with individual variation in fitness reflected in phenotypic differences. Resources must be acquired and allocated towards traits that increase current and future reproduction, and are often traded-off against survival. Fecundity selection is known to act through selection on traits that confer a fecundity advantage, and may be affected by a variety of extraneous factors. Chapter 6 examines the effect of direct selection for increased fecundity in female *P. interpunctella*. Correlated life-history traits known to increase/decrease fecundity are also examined, as well as early fecundity patterns.

Altering condition of animals can lead to some individuals developing more favourable phenotypes, which can affect their reproductive success. Diet is known to affect a number of traits that may positively or negatively influence male mating success, and/ or female fecundity. As sexes optimise their reproductive success in differing ways, this can lead to sexual conflict over diet choice. Chapter 7 examines the effect of diet on reproductive output in male and female *P. interpunctella*, a species under strong intralocus sexual conflict. The Geometric Framework is used to examine the impact of varying macronutrients on sex-specific differences in optimal diet for shared life-history traits (development time, longevity and body size), as well as survival rates.

2. Experimental Evolution Promotes Divergence in Shared Life-History Traits and Fitness in *Plodia interpunctella*.

2.1 Abstract

Males and females share much of the same genome, yet have different selective pressures in order to maximise their fitness. This can result in intralocus sexual conflict (IASC), where sexes are inhibited from evolving independently due to a genomic tug of war. Shared life-history traits have been found to be subject to opposing multivariate selection in the moth *Plodia interpunctella*, leading to evidence of strong IASC over fitness. We used replicate populations of *P. interpunctella* that have been evolving under varying adult sex ratios, reflecting different levels of sexual conflict. Adults were allowed to mate either in a female-biased (3:1 females: males), or male-biased (3:1 males: females) selection regime. Three life-history traits known to be related to reproductive success were measured (development time, longevity and body size), and fitness assays conducted. Evolved changes in life-history traits were recorded, and fitness between the diverged populations was found to differ. These results provide evidence of divergence in shared fitness related life-history traits between selection regimes. We discuss the implications for sexual dimorphism, trait evolution and possible sexual conflict resolution.

2.2 Introduction

Sexual conflict may arise when males and females invest differently towards reproductive strategies (Parker, 1979). We can classically think of this as males competing for access to females (Chapman *et al.*, 2003), and female's costly investment in producing large gametes (Trivers, 1972). There are two main forms of sexual conflict: interlocus and intralocus. Interlocus conflict (IRSC) occurs due to interactions of alleles at different loci between the sexes. This can lead to males having higher fitness optimums at the expense of females, and *vice versa* for females to show increased fitness at the expense of males (Chapman *et al.*, 2003). Conflicts are likely to occur over female mating rates, fertilisations, and parental effort (Arnqvist and Rowe, 2002). IRSC can cause the sexes to evolve counter-adaptations arising from reproductive interactions, leading to co-evolutionary arms races between the sexes (Parker, 1979).

Intralocus (IASC hereafter) arises due to the same alleles being expressed in both sexes but having opposite fitness effects. This occurs because many shared traits have a common genetic basis in the sexes, but are subject to opposing patterns of selection. These are known as sexually antagonistic alleles, as they are selected in opposite directions in the two sexes (Arnqvist and Rowe, 2002; Price and Hosken, 2007). If shared phenotypic traits are selected differently in the sexes, we may see one sex being driven away from its phenotypic optimum while the other sex shows increased fitness (van Doorn, 2009). This genomic tug-of-war may result in reduced population fitness as a whole, due to the gender load imposed by IASC (Arnqvist and Tuda, 2010). IASC is thought to be at its strongest when optimal trait values are

opposing in the sexes, and the intersexual genetic correlation (r_{mf}) for these traits is strong between males and females (Bonduriansky and Chenoweth, 2009).

Previous research has demonstrated the action of IASC in both wild and laboratory populations. Foerster *et al.* (2007) showed that in a wild population of red deer, high fitness males produced daughters of low fitness. Conflict has been shown to occur over immune defence between the sexes in side-blotched lizards, *Uta stansburiana* (Svensson *et al.*, 2009), as well as over diet choices in the field cricket, *Telogyllus commodus* (Maklakov *et al.*, 2008), with males and females being constrained in their optimal eating patterns. Human hip width is another suggested example, with the allele for small hips being favoured in men, which are optimal for walking. If this same allele is expressed in females it can lead to potentially harmful consequences during childbirth, leading to larger hips being favoured in females in order to minimise danger to the mother (Rice and Chippindale, 2001).

One thing most studies to date have in common is their use of univariate measures of selection to calculate differing fitness optima in the sexes without examining traits that may be driving these changes (Chippindale *et al.*, 2001; Fedorka and Mousseau, 2004; Pischedda and Chippindale, 2006; Foerster *et al.*, 2007). In the Indian meal moth, *Plodia interpunctella*, Lewis *et al.* (2011) demonstrated multivariate selection acting on three shared life-history traits (development time, longevity and body size) that are correlated with fitness. Selection acting on these shared life-history traits is shown to be opposing in the sexes, where a trait combination that favours females can be detrimental to males, and *vice versa*. This antagonistic selection is the signature of IASC (Arnqvist and Rowe, 2013). Life-history traits are likely to be subject to high levels of conflict, given their strong relationship to fitness, the fact they are

shared between the sexes, and have differing optima in the sexes (Wedell *et al.*, 2006). In male *P. interpunctella*, high fitness was optimised by a rapid development time and a long lifespan. Given that male fitness is dependent on achieving successful copulations, a rapid development time and longer lifespan would allow males to increase their chances of finding a mating partner, thus increasing the numbers of mating events (Lewis, 2005). Mating success in males has been shown to be unaffected by body size (Cook *et al.*, 1997). In contrast, female fitness was maximised by a long development time, shorter lifespan and a large body size. Large body size in female insects has been shown to increase fecundity, and is frequently traded off against lifespan (Honěk, 1993). This has a knock-on effect on development time, as more fecund females may need a longer period to acquire resources needed for egg production during the larval development stage (Nylin and Gotthard, 1998). It has been suggested that one way to overcome sexual conflict is through sexual dimorphism in the sexes (van Doorn, 2009; Pennell and Morrow, 2013). Recent studies have shown that despite the two sexes showing sexually dimorphic traits, IASC can remain strong and prevalent (Cox and Calsbeek, 2009; Harano *et al.*, 2010), as is the case for *P. interpunctella* (Lewis *et al.*, 2011).

Plodia interpunctella is an ideal species to study IASC, as conditions in the laboratory are very similar to conditions experienced in the wild being a pest of stored grains. This means that measures obtained for fitness and shared life-history traits in the lab would likely be reflected in its natural environment. *P. interpunctella* is a polyandrous species, with females mating around 1.79 times on average in their lifespan (Cook, 1999). *P. interpunctella* has shown responses to manipulation of adult sex ratio through experimental evolution due to increased risk of sperm competition

(Ingleby *et al.*, 2010). Males mated on average 0.77 times over a period of 3 days when evolving in a male biased adult mating regime, in contrast to mating 2.9 times in the female biased adult mating regime (Ingleby *et al.*, 2010). In comparison, females mate on average 2.3 times in the male-biased regime, in contrast to 0.97 times in the female-biased adult mating regime (Ingleby *et al.*, 2010). Evolved responses after altering adult mating sex ratio has been documented in other insects, such as *Drosophila melanogaster* (Wigby and Chapman, 2004), and the yellow dung fly (Hosken and Ward, 2001). The difference in number of mating events documented in *P. interpunctella* after a short time frame suggests that overall lifetime mating success may have diverged between the two sex-biased treatments. This is likely to be associated with correlated changes in shared life-history traits.

Altering the adult mating sex ratio by experimental evolution to be either male or female biased changes the selective forces between the sexes within populations. Conflict faced by sexes is likely to differ, due to increased competition for mates in the male biased treatment, or to a more relaxed selection regime with little male-male competition such as in the female biased treatment. As conflict usually stems from male competition for access to the limiting resource of females, and the choice females exert over their mating partners, conflict is expected to be most intense under a male biased sex ratio (Wigby and Chapman, 2004). Conversely, when females are abundant conflict may be reduced, and females less likely to suffer frequent mating attempts from males (Martin and Hosken, 2003; Wigby and Chapman, 2004).

A change in the adult mating sex ratio not only changes the conflict faced by sexes within the respective regimes, but also the opportunity for sexual selection (Jones, 2009). The intensity of sexual selection can be driven through pre-copulatory

mating system (Emlen and Oring, 1977; Andersson, 1994), with stronger selective forces on males from polygynous species compared with males from monogamous species (Wade and Shuster, 2004).

In this study we used experimental evolution to create replicate lines in which selection is expected to be stronger on the more abundant sex (Kvarnemo and Ahnesjö, 1996; Jones *et al.*, 2005). Given that IASC has been demonstrated in *P. interpunctella*, we aimed to create environments that would favour one sex at the expense of the other by altering the selection on adults. By manipulating adult mating sex ratio, we expect life-history trait divergence to evolve in favour of the more numerous sex. We predict that males in the male biased (MB) regime should evolve decreased development times and increased lifespan when compared to males from the female biased (FB) regime. Through evolution of favourable life-history trait combinations, we predict that males from the MB regime should have higher lifetime mating success (fitness) than males from the FB regime. Correspondingly, we predict that females from the FB regime should evolve life-history traits to increase lifetime fecundity, by taking longer to develop, having a larger body size and showing reduced lifespan when compared to females from the MB regime. We predict that females from the FB regime should evolve higher fecundity than females from the MB selection regimes.

2.3 Methods

2.3.1 Animal Husbandry

Two experimental evolution lines of *P. interpunctella* were utilised for this experiment. The only difference experienced in experimental evolution lines was the adult mating sex ratio; all populations experienced the same larval medium, reduced levels of larval competition and same temperatures and light cycles in the incubators. Larvae were reared on a diet of bran midlings, yeast, honey and glycerol, at a temperature of 28°C ($\pm 3^\circ\text{C}$) with a 16L: 8D photoperiod (Gage and Cook, 1994). Overcrowding was reduced by providing larvae with an excess of larval medium.

2.3.2 Experimental Evolution Populations

To obtain the experimental evolution populations of male biased (MB), and female biased (FB), adult *P. interpunctella* were allowed to mate under varying sex ratios. Adult moths only experienced changes in the adult mating competition, and larval development was standardised throughout. To obtain male biased population, adults were allowed to mate under a ratio of 3 males: 1 female. Larvae were randomly selected from each 1litre stock pot, and housed in same-sex stock pots, with approximately 120 males, and 40 females respectively. Larvae were easily sexed due to the males having their pigmented testes visible through dorsal side of their body cavity wall (Ingleby *et al.*, 2010). Upon eclosion into adults, males and females from these virgin stock pots were transferred into an egg collector. An egg collector consisted of an inverted 1litre pot with mesh fabric across the bottom. The inverted stock pot was placed in a funnel, which was attached to a conical flask. They were then allowed to mate for 3 days, with eggs being deposited at the bottom of the conical flask for ease of collection. These eggs were then used to establish a new generation. For the female

biased population, larvae were collected randomly, and allowed to mate under a ratio of 3 females: 1 male (i.e. 120 females: 40 males). For each experimental evolution selection line, there were 3 replicates giving a total of 6 populations maintained.

2.3.3 Life-History Traits

We measured the shared life-history traits (development time, longevity and body size) on individual males and females taken at random from each of the 3 replicate populations of MB and FB selection regimes. Life-history measures were conducted at around generation 85- 90. Male and female larvae were randomly selected and allowed to mate as adults under an equal sex ratio, to remove any potential maternal effects from both selection regimes. Eggs were collected every 24 hours, for a period of 3 days. A new conical flask was given each day, to ensure the eggs that were collected had been laid in the preceding 24 hour period. Eggs were transferred to a labelled 0.5 litre stock pot with an excess of larval medium inside. Once larvae had reached 4th instar, they were then transferred to individual 10-mL vials containing 4-mL of larval medium. As larvae reached 5th instar, vials were checked daily for eclosion in order to determine development time, measured as number of days between collection of egg and eclosion into adulthood. From day of focal animal eclosion, longevity was recorded as number of days until death. All focal animals were checked daily for survival. In total 30 males and 30 females from each population were examined.

Body size was estimated using a measurement of the right forewing of each experimental animal, which has previously been shown to correlate with overall size (Cook, 1996). Using a method featured in Reid (1976), the right forewing was

pulled carefully from the deceased moth using fine forceps. It was then dipped into 90% Isopropanol, followed by 10% hydrochloric acid to remove the scales. The wing was then immersed in thin bleach in order to remove the colour, before being washed in distilled water. The wing was then mounted onto a microscope slide, covered and left for 24 hours. Images of wings were captured using a Leica M125 microscope with camera attached, and measured using ImageJ software. The length between the junction of vein one and point of insertion was measured.

2.3.4 Fitness Assays

Males:

Male fitness was measured as lifetime mating success. From day of eclosion, each male was allocated a single, virgin female from within the same population every 24 hours. Each morning before scotophase, the previous day females were removed, placed in labelled Eppendorf according to focal male, and frozen at -20°C. Successful copulations were confirmed by dissection of all females, to ensure spermatophore transfer had taken place. Once sperm has drained from the spermatophore, the remnants remain inside the bursa, which can then be counted in order to determine if a male successfully copulated with a female (Drummond, 1984). This process was continued for the lifetime of the male. Each female was placed with the male for a 24 hour period, so all males were subjected to constant virgin female access.

Females:

Female fitness was measured as lifetime fecundity. On day of eclosion, each female was mated once to a single, virgin male from within the same population. After

24 hours, the female was then removed and placed into an individual 10-mL vial modified for egg collection. Due to females preferring to oviposit in the presence of larval medium, a small amount of food was placed at the bottom, separated from the female by a cone of filter paper. This helped to stimulate oviposition, and eggs were collected in the cone of the filter paper. Modified caps were replaced every 48 hours, allowing eggs to be counted for remainder of the female's life.

Focal insects were mated from within the same population allowing us to measure fitness levels under the conditions moths have been evolving in the laboratory. Standardisation could be achieved, ensuring mates were of same age or younger than focal moths, that reduces the risk of biasing results due to lack of attractiveness. Previous work has shown that mating from within or between the selection regimes had no effect on reproductive output (Lewis, unpublished data).

2.3.5 Egg Fertility

Fertility of eggs was conducted using the same female and male biased selection regimes already established in the laboratory. Approximately 30 female and male 5th instar larvae were randomly selected from each of the six replicate populations around generations 100- 105. Larvae were housed in single-sex pots to ensure virginity upon eclosion. When adults moths eclosed, virgin focal females were paired with a randomly selected virgin male no older than 3 days old. After 24 hours, the males were removed whilst the focal females remained in the Sterlin vial. A modified egg collector was given to focal females. Eggs were counted 72 hours, and transferred into a small petri dish containing double-sided sticky tape. This ensured that hatched larvae could not escape, or cannibalise each other, so numbers could be recorded. Petri dishes were sealed with Parafilm before being placed back inside the

incubator. Fertility was recorded as number of larvae that had successfully hatched from the eggs after a further four days had passed. Focal females were frozen at -20°C in order to determine body size using measure of right wing length.

2.3.6 Statistical Analysis

Effects of Selection Regime and Sex:

A multivariate analysis of variance (MANOVA) was used to test for differences in the shared life-history traits (development time, longevity and body size). Independent variables in the model were: sex, sex ratio treatment, and the interaction between sex and treatment. The effect of replicate was nested within treatment. Minimal adequate model was achieved by removing a non-significant interaction between sex and treatment regime. Further analysis using post-hoc analysis of variance (ANOVA) was then used to test for differences in life-history traits between the sexes. Dependant variables were checked for normality by visual inspection of residuals and QQ plots. Any extreme outliers (greater than 2.5 SD away from mean) were removed. A total of 12 outliers were removed (longevity: 2 females and 2 males from FB, and 3 males from the MB; Body size: 2 females from FB and 3 females from the MB).

Life-History Traits between Selection Regimes:

To analyse the differences in shared life-history traits within the sexes across the treatment regimes, we analysed males and females separately. A MANOVA was conducted as before, with post-hoc ANOVA's for each of the life-history traits to see if the effect of sex ratio regime differed within the sexes.

Fitness between Selection Regimes:

As fitness was not measured in the same way for both males and females, we split the data according to sex. Male lifetime mating success was non-normally distributed, and could not be transformed to normality. Generalised linear model (GLM) with poisson error distribution was used to test for differences in fitness between males of the MB and FB line respectively. Four outliers for mating success were removed as they were more than 2.5 SD away from the mean (2 from FB and 2 from MB). Effect of replicate was tested for by nesting within selection regime.

Female lifetime fecundity was found to be normally distributed and an ANOVA was used to compare differences between the selection regimes. Effect of replicate was tested for by nesting within selection regime.

Fertility between Selection Regimes

Proportion of eggs hatching was non-normally distributed, so a generalised linear model (GLM) was used to test for differences in fertility of eggs between the FB and MB treatments respectively. Effect of replicate was tested for, and body size was placed in the model as a covariate.

All statistical measures were conducted using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

2.4 Results

2.4.1 Effects of Selection Regime and Sex

Selection regime was found to have a significant effect on the shared life-history traits (Table 2.1). Moths within the MB selection regime were quicker to

develop on average than those in the FB selection regime across both sexes (Table 2.1). Body size was found to be larger on average in moths from the MB compared to FB selection regimes (Table 2.1). Longevity did not significantly differ overall between the two selection regimes (Table 2.1). Replicate was found to be significant for all shared life-history traits in *P. interpunctella* (MANOVA; Pilla's Trace = 0.879, $F_{12, 1014} = 35.012$, $P < 0.001$); development time ($F_{4, 338} = 343.278$, $P < 0.001$; Replicate \pm SE FB1: 33.18 ± 0.16 ; FB2: 36.63 ± 0.09 ; FB3: 37.39 ± 0.12 ; MB1: 37.03 ± 0.10 ; MB2: 32.98 ± 0.09 ; MB3: 32.69 ± 0.17 days), longevity ($F_{4, 338} = 4.196$, $P = 0.002$; Replicate \pm SE FB1: 6.72 ± 0.18 ; FB2: 6.73 ± 0.19 ; FB3: 7.19 ± 0.18 ; MB1: 7.20 ± 0.15 ; MB2: 7.24 ± 0.17 ; MB3: 6.59 ± 0.19 days), and body size ($F_{4, 338} = 4.48$, $P = 0.002$; Replicate \pm SE FB1: 4.20 ± 0.05 ; FB2: 4.05 ± 0.05 ; FB3: 4.24 ± 0.06 ; MB1: 4.39 ± 0.06 ; MB2: 4.37 ± 0.05 ; MB3: 4.28 ± 0.05 mm).

Sex was found to have a significant effect on two of the three shared life-history traits, indicating sexual dimorphism in this species within both selection regimes (MANOVA; Pilla's Trace = 0.58, $F_{3, 336} = 154.709$, $P < 0.001$). Males lived longer than females on average ($F_{1, 338} = 123.96$, $P < 0.001$; Males = 7.614 ± 0.086 days; Females = 6.253 ± 0.087 days). Females had a larger body size on average in comparison to males ($F_{1, 338} = 276.615$, $P < 0.001$; Males mean ($\pm 1SE$) = 3.998 ± 0.021 mm Females = 4.487 ± 0.021 mm). In contrast, there was no significant difference in development time between the sexes ($F_{1, 338} = 1.187$, $P = 0.277$; Males mean ($\pm 1SE$) = 34.925 ± 0.073 days; Females = 35.037 ± 0.073 days).

Table 2.1: MANOVA examining differences in shared life-history traits (development time (DT), longevity and body size) across the female-biased (FB), and male-biased (MB) selection regimes. Means (± 1 SE) are included for each of the life-history traits for both selection regimes. Significant P values are highlighted **bold**.

MANOVA			
Selection	Pilla's Trace	$F_{(3,336)}$	P
	0.413	78.937	< 0.001

ANOVA				
	MB	FB	$F_{(1,338)}$	P
DT (days)	34.235 \pm 0.073	35.727 \pm 0.073	209.753	< 0.001
Longevity (days)	7.019 \pm 0.086	6.849 \pm 0.086	1.938	0.172
Body size (mm)	4.320 \pm 0.021	4.165 \pm 0.021	27.858	< 0.001

2.4.2 Male life-history traits

Selection regime had a significant effect on all three life-history traits in males (MANOVA; Pilla's trace = 0.491, $F_{3,165} = 53.095$, $P < 0.001$), with males from the MB selection regime having shorter development times ($F_{1,167} = 145.848$, $P < 0.001$), and living longer on average compared to males from the FB regime ($F_{1,167} = 5.629$, $P = 0.019$). Comparison of body size between the two selection regimes revealed that males in the MB are larger than males in the FB selection regime ($F_{1,167} = 22.825$, $P < 0.001$). See Table 2.2 for overall means of life-history traits for males within the two selection regimes. The effect of replicate was found to be significant on male shared life-history traits (MANOVA; Pilla's Trace = 1.007, $F_{12,501}$, $P < 0.001$); development time ($F_{4,167} = 227.844$, $P < 0.001$; Replicate \pm SE FB1: 33.07 \pm 0.19; FB2: 36.70 \pm 0.14; FB3: 37.37 \pm 0.17; MB1: 37.00 \pm 0.14; MB2: 32.83 \pm 0.12; MB3: 32.59 \pm 0.19 days), longevity ($F_{4,167} = 6.104$, $P < 0.001$; Replicate \pm SE FB1: 6.76 \pm 0.21; FB2: 7.60 \pm 0.23; FB3: 8.03 \pm 0.16; MB1: 7.76 \pm 0.16; MB2: 8.07 \pm 0.17; MB3: 7.59 \pm 0.26 days) and body size ($F_{4,167}$

= 3.02, $P = 0.019$; Replicate \pm SE FB1: 3.96 ± 0.05 ; FB2: 3.80 ± 0.03 ; FB3: 3.97 ± 0.04 ;
 MB1: 4.11 ± 0.05 ; MB2: 4.12 ± 0.05 ; MB3: 4.02 ± 0.05 mm).

Table 2.2: Means (\pm 1 SE) for life-history traits (development time (DT), longevity and body size) for both males and females within male-biased (MB), and female-biased (FB) selection regimes.

	Males		Females	
	MB	FB	MB	FB
DT (days)	34.14 ± 0.093	35.709 ± 0.091	34.328 ± 0.113	35.745 ± 0.116
Longevity (days)	7.807 ± 0.115	7.425 ± 0.113	6.244 ± 0.121	6.271 ± 0.124
Body size (mm)	4.084 ± 0.026	3.912 ± 0.025	4.556 ± 0.033	4.418 ± 0.034

2.4.3 Female life-history traits:

Selection regime was found to have a significant effect between females of the MB and FB regime for two of the three life-history traits (MANOVA; Pilla's trace = 0.348, $F_{3,164} = 29.168$, $P < 0.001$). Females in the FB selection regime had longer development times ($F_{1,166} = 76.847$, $P < 0.001$), and significantly smaller body size on average ($F_{1,166} = 8.568$, $P = 0.004$) compared to females from the MB regime. There was no significant effect of selection regime on longevity ($F_{1,166} = 0.026$, $P = 0.872$). See Table 2.2 for overall means of life-history traits for females within their respective selection regimes. Replicate was found to have a significant effect within selection regimes (MANOVA, Pilla's trace = 0.348, $F_{3,164} = 29.168$, $P < 0.001$); development time ($F_{1,166} = 76.847$, $P < 0.001$; Replicate \pm SE FB1: 33.29 ± 0.26 ; FB2: 36.57 ± 0.11 ; FB3: 37.41 ± 0.18 ; MB1: 37.07 ± 0.14 ; MB2: 33.13 ± 0.13 ; MB3: 32.77 ± 0.28 days) and body size ($F_{1,166} = 8.568$, $P = 0.004$; Replicate \pm SE FB1: 4.45 ± 0.05 ; FB2: 4.31 ± 0.06 ; FB3:

4.53 ± 0.08; MB1: 4.66 ± 0.08; MB2: 4.61 ± 0.05; MB3: 4.50 ± 0.05 mm) but not for lifespan ($F_{1, 166} = 0.026, P = 0.872$).

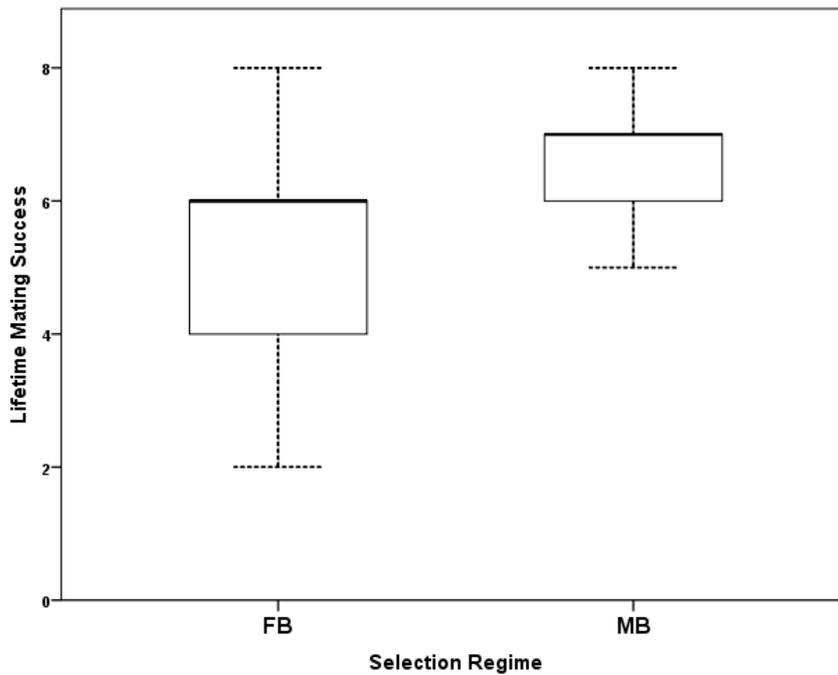


Figure 2.1: Median lifetime mating success of males from female biased (FB) and male biased (MB) selection regimes. Males in the MB achieved significantly more successful copulations than males in the FB line. Interquartile ranges are also shown.

2.4.4 Male Fitness

There was evidence that fitness had diverged between the selection regimes, with males from the male biased (MB) populations achieving increased lifetime mating success compared to males in the female biased (FB) regime (GLM; Wald $\chi^2_1 = 5.369, N = 163, P = 0.02$; Figure 2.1). There was a significant effect of replicate within selection regimes (Wald $\chi^2_4 = 10.781, N = 163, P = 0.029$; Replicate ± SE FB1: 4.24 ± 0.26; FB2:

5.93 ± 0.26; FB3: 5.73 ± 0.27; MB1: 6.00 ± 0.25; MB2: 6.59 ± 0.26; MB3: 5.74 ± 0.27

lifetime mating success).

2.4.5 Female Fitness

In contrast, females from the female biased (FB) selection regime laid significantly fewer eggs over the course of their lifespan compared to females from the male biased (MB) regime (ANOVA; $F_{1,166} = 5.172$, $P = 0.024$; FB means ± 1 SE = 132.481 ± 5.622, MB = 150.347 ± 5.488; Figure 2.2). Replicate was found to have a marginally non-significant effect within selection regimes ($F_{4,166} = 2.399$, $P = 0.052$).

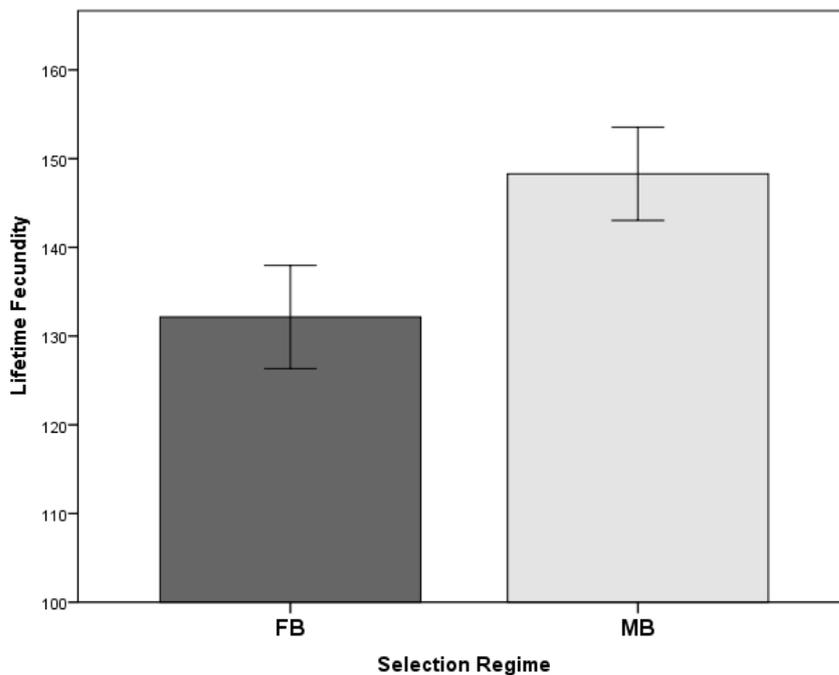


Figure 2.2: Mean lifetime fecundity in females from female-biased (FB; dark grey bar) and male-biased (MB; pale grey bar) selection regimes. FB females were less fecund than those from the MB selection regime. Error bars indicate ± 1 SE.

2.4.6 Fertility of Eggs

The proportion of hatched eggs was significantly different between selection regimes, with females from the MB line showing higher fertility when compared to females from the FB regime (GLM; Wald $\chi^2_1 = 69.2$, $N = 120$, $P < 0.001$; Figure 2.3).

There was no significant effect of replicate within selection regime (GLM; Wald $\chi^2_4 = 5.259$, $N = 120$, $P = 0.26$), and covariate body size had no significant effect on female fertility (GLM; Wald $\chi^2_1 = 0.339$, $N = 120$, $P = 0.56$).

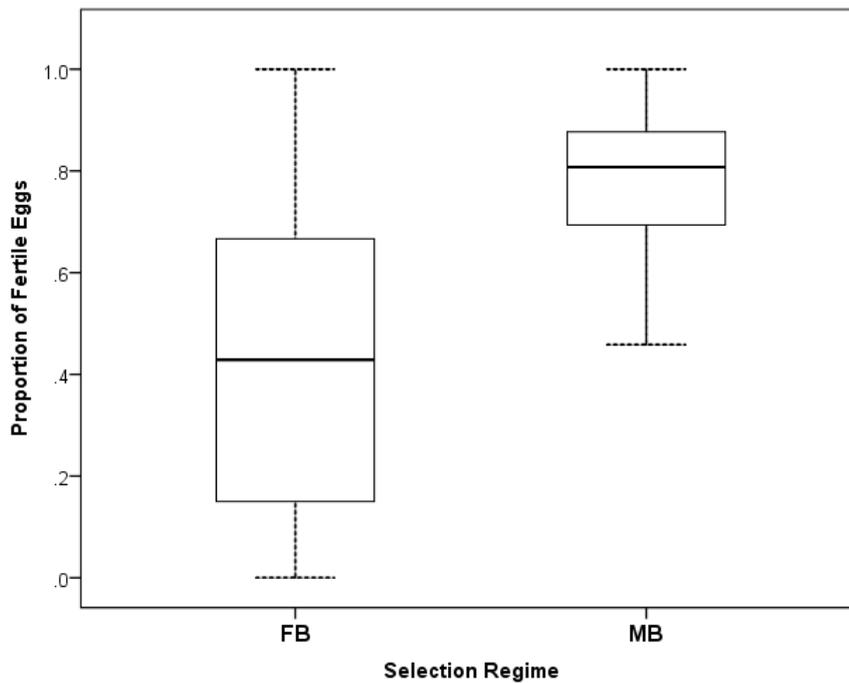


Figure 2.3: Boxplot showing the fertility (proportion of hatched eggs) of females from the female-biased (FB) and male-biased (MB) selection regimes. Females in the MB had a higher proportion of eggs hatch successfully than females in the FB line. Inter-quartile ranges are also shown.

2.5 Discussion

Altering selection by varying adult mating sex ratios in *Plodia interpunctella* led to fitness divergence between populations. Males had higher mating success in the male biased (MB) regime, with corresponding life-history trait changes. As predicted, males in the MB had slower development times, and longer lifespans compared to males in the female biased (FB) regime. In contrast to our predictions, we found females in the FB regime had lower lifetime fecundity relative to MB females. Lower fitness measures in the FB females were also reflected through smaller body size, longer development times, but no difference in lifespan compared to MB females.

2.5.1 Comparisons of Males between Selection Regimes

Males in the MB selection regime had higher mating success when compared to males from FB regime, with MB males securing one additional copulation on average over the course of their life (Figure 2.1). We predicted that as fitness increases for males in MB regime, there would also be corresponding changes to life-history traits (development time, longevity and body size), with longer lifespans and shorter development times being favoured. In line with our predictions, males in MB regime had increased longevity compared to males in the FB selection regime (Table 2.2). Longer lifespans are advantageous to males as it allows a longer time frame in which to secure successful copulations due to increased encounter rate (Lewis, 2005). Development time is also found to be significantly shorter for males in the MB regime when compared to their male FB conspecifics. Developing and eclosing earlier is likely to allow males a greater opportunity to achieve a successful copulation (Lewis, 2005; Lewis *et al.*, 2011). Males in the MB regime were significantly larger in body size

compared to FB males (Table 2.2). Pre-copulatory mechanisms may favour large body size, such as male-male competition and female mate choice. However, whilst we measured mating success without male-male competition, we examined the possibility of larger males securing more copulations by looking at the relationship between male body size and lifetime mating success. Whilst we found a slight positive correlation of body size on mating success overall on all males used in experiment (Pearson's correlation; $r_{(173)} = 0.155$, $P = 0.042$), this pattern was not present for males within the MB regime (Pearson's correlation; $r_{(85)} = 0.106$, $P = 0.334$), suggesting that large male size is not selected for via increased pre-copulatory success, measured as lifetime mating success, in the MB populations. This corroborates previous work in *P. interpunctella*, finding no clear relationship between male body size and mating success (Cook *et al.*, 1997). Female mate choice may favour large male size, as they may donate bigger nutritious spermatophores (Fritzsche and Arnqvist, 2015). In addition, larger males may be better at courting/ seducing females (Maklakov *et al.*, 2004). Males in the MB treatment experience increased risk and intensity of sperm competition compared to males in the FB regime. Selection should favour adaptations that are advantageous in increasing male paternity success in sperm competition (Parker, 1970b). Ingleby *et al.* (2010) demonstrate that males from the very same MB regime consistently produce and donate more sperm compared to FB males, which may translate to higher paternity in sperm competition. Furthermore, males in the MB show higher paternity success in P₁, sperm defence role, relative to FB males (Chapter 3). Males' ability to defend their investment from a rival male's ejaculate is increased, allowing sperm precedence and higher paternity success in P₁ role. However, Cook *et al.* (1997) found no effect of male size on P₂ (sperm offence) in *P. interpunctella*. In other species of Lepidoptera, larger body size is shown to positively correlate with

amount of ejaculate produced (Bissoondath and Wiklund, 1996; Wedell and Cook, 1999). Larger male size may be favoured in sperm competition due to males producing larger spermatophores (Wedell, 1997). In summary, males evolving under male-biased adult sex ratio show increased lifetime mating success, and corresponding changes to shared life-history traits as predicted under the selection regime.

2.5.2 Comparisons of Females between Selection Regimes

In contrast, we found that females in the FB regime did not show higher lifetime fecundity as predicted, with FB females laying almost 18 eggs less over the course of their lifespan than MB females (Figure 2.2). Fertility of eggs laid by females in the FB regime is lower, as a smaller proportion of eggs successfully hatch compared to MB females (Figure 2.3). We predicted that as female fitness increases in FB regime, there would be corresponding changes in life-history traits, with larger body size, longer development time and shorter lifespan being favoured. However, while we see life-history traits changes in relation to the lower fecundity measures for females in FB regime they are not all in the predicted direction. FB females show smaller body size, no difference in lifespan, and longer development times compared to the high fecundity MB females. Body size differences may drive these low fecundity measures in females from the FB selection regime, as they were significantly smaller on average to females in the MB lines. As larger body size in female insects is known to be positively correlated with fecundity (Honěk, 1993, and Blanckenhorn, 2000), this may relate to the differences in fecundity we observed here. Despite development taking a longer time in females from the FB, there is no correlated fecundity response in female fitness. Longer development is predicted to be favourable for females when all energy

accrued for reproduction is at the larval stage, as is the case for this semelparous species (Trivers, 1972; Gage, 1995). Despite fecundity differences, there was no evident trade-off with lifespan, as we found no significant differences in lifespan between females of the FB and MB regime. Lifespan is often frequently traded off against reproduction in female insects (Chapman *et al.*, 1998). In summary, we did not find that females in the FB regime had higher fecundity than females in the MB regime. As a corollary, we also find that there are changes in life-history traits for FB females that support lower fecundity response.

Another possible reason for reduced fitness levels in the FB selection regime may be due to inbreeding depression. Inbreeding can lead to lowered optimal phenotypes within populations, by increasing the exposure of deleterious recessive alleles (Pusey and Wolf, 1996). However, we think this is unlikely to be the case for the FB regime. Both males and females in the FB populations mate at least once, compared to most males in MB regime who mate less than once on average over 3 days (Ingleby *et al.*, 2010). Under a competitive mating regime replicating adult mating sex ratios, it has been found that only 17% of males in the MB secure a successful mating, leading to larger variation in mating success within MB populations (Larner *et al.*, unpublished data). As a consequence a higher number of individuals in the FB regime contribute to the next generation, relative to the MB regime.

2.5.3 Comparison within Selection Regime

Three shared life-history traits (development time, body size and longevity) are known to be genetically correlated between the sexes in *P. interpunctella*, and are subject to sexually antagonistic selection (Lewis *et al.*, 2011). Due to sexually

antagonistic alleles, as fitness in one sex is increased, the other sex may be pulled away from its phenotypic fitness optimum (Bonduriansky and Chenoweth, 2009). We therefore predicted that males in the MB regime should have higher mating success, with correlated changes in life-history traits. We also predicted that this change would be at the expense of the females in the same MB selection regime, with females having lower fecundity and corresponding changes in life-history traits (e.g. smaller body size, longer lifespan and reduced development time). However, we found in fact that MB females had higher lifetime fecundity than females evolving in the FB regime (Figure 3.2). Furthermore, there were corresponding changes in shared life-history traits that may support the observed higher fecundity. On average, larger body sizes, reduced development times, and no significant differences in lifespan were observed in females in the MB compared to females in FB regime. Both males and females in the MB regime have diverged in shared life-history traits that result in increased fitness measures for both sexes relative to FB moths.

In contrast, in the FB regime we observed lower reproductive success in both sexes. We predicted that fecundity in the FB line should increase as a result of stronger selection on females, which would be at expense on their male conspecifics. Yet we find lower fecundity for the former and fewer successful copulations for males. Changes in shared life-history traits in females, such as smaller body size, may translate to the lower fecundity recorded in FB line females. Males in FB regime also show associated changes in shared life-history traits, such as increased development time, reduced lifespan and smaller body size that in combination may result in lower mating success. Both sexes in the FB regime show changes in shared life-history traits associated with lower lifetime fitness compared to moths in the MB selection regime.

These experimental evolution populations were examined as selection was predicted to be stronger on the more numerous sex (Kokko and Jennions, 2008). We expected that males in the MB regime would be under stronger selection than their female conspecifics, and *vice versa* we expected females in the FB regime to be subject to stronger selection than males in the same regime. However, selective forces may not be equal across the two sex-biased treatments, and may generate differential rates of change in the moth populations. In other words, evolving under a male-biased sex ratio may translate to stronger overall selection on males than the selection experienced by females evolving under a female-biased sex ratio. Intralocus sexual conflict (IASC) is a transient force, and liable to change over evolutionary time within populations (Cox and Calsbeek, 2009). Bonduriansky and Chenoweth (2009) identify and discuss four phases of IASC, *i*) before IASC, *ii*) acute IASC, *iii*) attenuated IASC and *iv*) resolved IASC (see Figure 3.4). Phases *i* and *iv* are representative of stabilising selection acting on the traits in question and resulting fitness. This results in the intersexual additive genetic correlation, r_{mf} , being equal to 1. r_{mf} can be calculated for a population by measuring additive genetic variation in both sexes for a trait (Bonduriansky and Chenoweth, 2009). These authors argue that sexual conflict resolution is achieved in phase *iv*, (see Figure 3.4), when both sexes diverge towards optimal sexual dimorphism, which results in increased fitness for both sexes as sexual antagonism is diminished. Phase *ii* and *iii* are more akin to $r_{mf} < 0$, when trait values and fitness are opposing between the sexes. As we see in Figure 3.4, phase *iii* is representative of IASC abating, and both sexes start to diverge towards their respective sex- specific fitness optima. This may be the case for moths in the MB regime. Increased fitness and correlated changes in shared life-history traits suggest that conflict resolution may be under way in the MB selection regime. In contrast, the

FB selection regime show lower fitness in both sexes compared to the MB selection regime. A gender load, where adaptation in one sex is consistently constrained by sexually antagonistic selection in the other sex, may be imposed due to IASC, and can reduce the fitness of a population (Long *et al.*, 2006; Arnqvist and Tuada, 2010). This may correspond to phase *ii*, acute IASC, where both sexes are displaced from their phenotypic optimums.

Sexual dimorphism has been suggested as a way for the sexes to overcome intralocus sexual conflict, IASC (Lande, 1980). However, further research demonstrates that populations can still have IASC whilst simultaneously being sexually dimorphic (Harano *et al.*, 2010). This also appears to be the case for *P. interpunctella* as there is evidence of strong IASC, despite the sexes having sexually dimorphic traits (Lewis *et al.*, 2011). We examined the degree of sexual dimorphism, measured as difference in mean shared life-history traits, between the sexes across all 3 populations within each selection regime (Δ Males - Females; Table 3.3). Development time and longevity show a greater degree of sexual dimorphism in life-history traits between males and females in the MB regime compared to moths in the FB regime (Table 3.3). MB moths show more pronounced sexual dimorphism than FB moths. Both sexes diverge towards optimal fitness phenotypes as sexual antagonistic selection and fitness variation subsides (Bonduriansky and Chenoweth, 2009). With smaller degree of sexual dimorphism being observed in shared life-history traits for moths in the FB regime, we can speculate that both sexes are still under sexually antagonistic selection, with neither sex having reached their sex-specific fitness optima.

Table 2.3: Mean shared life-history traits (development time (DT), longevity and body size) for both males and females of the male-biased (MB) and female-biased (FB) selection regimes. Difference (Δ) between the sexes within each selection regime is also shown. Greatest degree of sexual dimorphism between sexes is highlighted in **bold**.

	MB			FB		
	Males	Females	Δ	Males	Females	Δ
DT (days)	34.140	34.325	-0.185	35.712	35.753	-0.041
Longevity (days)	7.807	6.270	1.537	7.464	6.268	1.196
Body size (mm)	4.084	4.588	-0.504	3.911	4.430	-0.519

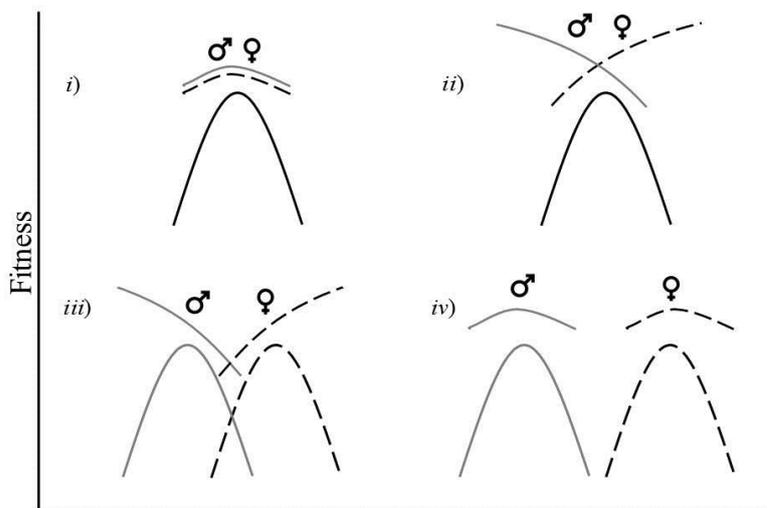


Figure 2.4: Phases of IASC. Phase *i*) Before IASC - shared trait under weak, stabilising selection in both sexes (males: grey solid line; females: dashed black line), with mean fitness distribution (solid black curve). Phase *ii*) Acute IASC - strong sexually antagonistic selection acting on shared trait, displacing sexes away from their phenotypic optimum. Phase *iii*) Attenuated IASC - sexually antagonistic selection on shared trait starts to abate, and sexes begin to evolve sexual dimorphism. Phase *iv*) Resolved IASC - optimal sexual dimorphism evolves, as sexual antagonism diminishes with sexes under stabilising selection. (Redrawn from BONDURIANSKY and CHENOWETH, 2009)

Finally, a non-mutually exclusive explanation is that sexual selection is likely acting within the diverged selection regimes, at differing strengths. Whenever reproduction occurs, sexual selection will operate to varying degrees (Andersson, 1994). This may not only affect individual fitness, but population fitness as a whole. Increased mutation loads may build up in populations as natural selection may not target small effects caused by deleterious alleles at the individual level (Agrawal *et al.*, 2012). Sexual selection is proposed to purge deleterious alleles, and cause fixation of advantageous ones (Siller, 2001). A recent study examined the effects of varying sexual selection intensities in the flour beetle, *Tribolium castaneum* showing that sexual selection can increase population viability (Lumley *et al.*, 2015). Extinction rates following inbreeding were used as a proxy of the level of deleterious alleles present within *T. castaneum* populations that had either been evolving under varying levels of sexual selection (biased sex ratios (MB vs FB), or under polyandry (female multiple mating) vs monogamy (single female mating)). Beetles that had descended from male biased and polyandrous populations were 40% more resilient to extinction, compared to female biased and monogamous populations (Lumley *et al.*, 2015). This finding indicates that sexual selection has filtered out deleterious alleles, allowing higher fitness measures to be observed. It is possible increased opportunity for sexual selection may also partly explain our finding of higher fitness overall for both sexes within the MB regimes. Both MB male and female *P. interpunctella* had increased reproductive success, and egg fertility was significantly higher than moths from the FB regimes. Sexual selection is a potent force potentially driving male mating success, and increasing female lifetime fecundity. Sexual conflict, as well as sexual selection may therefore act in tandem together within our experimental evolution *P. interpunctella* populations, but where the effects of one being hard to disentangle from the other.

2.5.4 Conclusion

Altering selection through biasing the adult mating sex ratio causes divergence in both fitness and corresponding shared life-history traits in evolving *Plodia interpunctella* populations. We demonstrate that males in the MB regime have evolved life-history traits associated with increased fitness relative to FB males. Remarkably, this has not been at the expense of the females within the same MB regime, despite shared life-history traits showing strong intralocus sexual conflict (Lewis *et al.*, 2011). It is possible this may be due to resolution of IASC within the MB selection regime by promoting greater sexual dimorphism in life-history traits that are optimal for both sexes, potentially due to experiencing stronger selection than in the FB selection regime. In comparison, moths in the FB may be suffering a gender load resulting in lower fitness for both sexes. There is a smaller degree of sexual dimorphism in the examined life-history traits between sexes in the FB regime. These results suggest that sexually antagonistic selection may be inhibiting sexes from achieving optimal phenotypes in the FB treatment. Sexual selection and conflict are both powerful forces, with the ability to change the evolutionary trajectory of a species over time.

3. Relationships between traits associated with sperm defence and offence in the moth, *Plodia interpunctella*.

3.1 Abstract

Intensity and risk of sperm competition faced by males shape ejaculate evolution, and ultimately a males' reproductive success under post-copulatory selection. Using the sterile male technique we measured sperm competitiveness, quantified as male sperm defence (P_1), and sperm offence (P_2) ability in the Indian meal moth, *Plodia interpunctella*. By biasing the adult mating sex ratio, we altered the levels of sperm competition faced by males, showing that males were selected to increase traits with sperm defence when evolving under increased risk and intensity of sperm competition. We found no evidence of a relationship between P_1 and P_2 suggesting that sperm mixing in *P. interpunctella* is not random. We also show that males adopt differing strategies depending on levels of sperm competition faced, but ultimately sire equal numbers of offspring within their respective treatments.

3.2 Introduction

In species where females mate multiply and store ejaculates of multiple males, sperm competition is generated over fertilisation of the ova (Parker, 1970b). Males can maximise their post-copulatory reproductive success through defensive adaptations that protect the ejaculate after insemination against a rival male, as well as through offensive adaptations to overcome a rival males' ejaculate when mating with an already inseminated female. Theory predicts antagonistic selection between defensive and offensive traits if there are different costs associated with each strategy (Parker,

1984). A male may assume differing mating roles while engaged in sperm competition, mating either in defensive (first male) or offensive (second/ last male) roles. In order to determine a male's post-copulatory success, paternity shares can be measured when mating in the first role (P_1 , defensive) or second male role (P_2 , offensive) (Boorman and Parker, 1976). Selection may act independently on each of these male roles, as traits which increase P_1 may not increase P_2 , and vice versa (Parker, 1984). A fair raffle of sperm competition assumes that each male will have a proportionate share of paternity based on the number of sperm donated to the female at mating (Parker, 1990). This suggests that in species where sperm competition operates, and the quantity of sperm delivered determines sperm competitiveness, it is likely that P_1 and P_2 will be correlated and both contribute towards reproductive fitness (House and Simmons, 2006). This may not be true in species with ejaculates that contain non-sperm components that influence the outcome of sperm competition (Chapman, 2001; Clark *et al.*, 2005), or in species where defensive and offensive ejaculate traits evolve independently (Clark *et al.*, 2005). There may also be nontransitive interactions between different male's ejaculates, or between males and females (Bjork *et al.*, 2007). In *Drosophila melanogaster*, a species with second male precedence, it has been shown that P_2 , but not P_1 , correlated with male lifetime reproductive success (Fricke *et al.*, 2010), and is likely that in species with strong sperm defence ability the opposite may be true. There are many empirical studies in insects showing clear patterns of male sperm precedence (reviewed in Simmons, 2001). However, general relationships between male sperm defence and offence ability are unclear, with studies finding positive, negative or no correlations between P_1 and P_2 (Bernasconi and Keller, 2001; Friberg *et al.*, 2005; House and Simmons, 2006; Fricke *et al.*, 2010).

Theory predicts that as the intensity (how many ejaculates are competing over eggs) and risk (likelihood of female remating) of sperm competition increase, selection will favour adaptations that are advantageous in increasing males' paternity share (Parker, 1970b). Empirical demonstrations in the dung fly *Scathophaga stercoraria* (Hosken *et al.*, 2001) and the Indian meal moth *Plodia interpunctella* (Ingleby *et al.*, 2010), show that males evolving under higher sperm competition evolve to produce bigger ejaculates, and the same patterns is also found across taxa as revealed by comparative studies (Møller, 1988; Hosken, 1997; Byrne *et al.*, 2002). When risk of sperm competition is high males have been shown to strategically allocate sperm (Cook and Wedell, 1996; Ingleby *et al.*, 2010) and to adjust the size of ejaculates (Simmons *et al.*, 1999). Sperm production can be costly (Wedell *et al.*, 2002; Hayward and Gillooly, 2011), and so males are expected to tailor their ejaculate to maximise reproductive returns (Drewsbury, 1982). As the intensity and risk of sperm competition differs, so may the selection pressures that act on traits associated with P_1 and P_2 (Bernasconi and Keller, 2001). Understanding the relationships between sperm defence and offence abilities, how these may differ with intensity of sperm competition and how they relate to male reproductive success may provide insights to the patterns of selection.

Intensity and risk of sperm competition may be altered by exposing males for multiple generations to varied mating conditions, such as polyandrous and monandrous treatments (Hosken *et al.*, 2001), or differing adult sex ratios (Wigby and Chapman, 2004; Ingleby *et al.*, 2010). When a mating population is male-biased, females are likely to mate with multiple males, increasing the risk of sperm competition and possibly also the intensity faced by males. Experimental evolution has been used to measure the consequence of increased risk and intensity of sperm

competition in a number of species, for example the fruit fly *D. melanogaster* (Wigby and Chapman, 2004) and yellow dung flies *S. stercoraria* (Hosken *et al.*, 2001). Male *P. interpunctella* has shown responses to manipulation of adult sex ratio through experimental evolution in response to increased risk of sperm competition (Ingleby *et al.*, 2010). Males evolving under male-biased adult sex ratios where females mated on average 2.3 times consistently donated larger ejaculates in contrast to the female-biased populations where females mated 0.97 times in a period of 3 days (Ingleby *et al.*, 2010). These studies have shown that males evolved increased investment in sperm production as a response to increased intensity and risk of sperm competition.

In order to measure P_1 and P_2 values, paternity must be assigned to offspring. This can be done using morphological traits (Bernasconi and Keller, 2001; Fricke *et al.*, 2010), DNA markers (Evans *et al.*, 2003) or by using the sterile male technique (Parker, 1970b; Boorman and Parker, 1976; House and Simmons, 2006; Harano *et al.*, 2008). The sterile male technique uses the lowest dose of radiation or chemical to treat males that induces mortality of all offspring sired before hatching and then assigning paternity by hatch rate of eggs. Eggs that fail to hatch are assigned to the treated male and eggs that hatch assigned to the normal male (Parker, 1970b). A recent study by Xu and Wang (2010a) used chemo-sterilisation by thiotepa to determine sperm precedence in the moth, *Ephesia kuehniella*. Thiotepa is an effective sterilant of insects and has been shown to have no adverse effects to mating, longevity (Nabi and Harrison, 1984a, b; Xu and Wang, 2010a) or sperm competitiveness.

A unique feature of Lepidopteran males is the production of two types of sperm: non-fertile “apyrene” sperm, and fertile “eupyrene sperm (Friedländer, 1997). Despite not being able to fertilise eggs, apyrene sperm travel faster to the spermathecal than fertile sperm (Watanabe *et al.*, 2000), as well as make up to 95% of

an ejaculate (Cook and Wedell, 1996). This suggests that apyrene sperm may have an adaptive value to Lepidopterans, and are proposed to fill the spermatheca in order to delay remating (Silberglied *et al.*, 1984; Wedell, 2005). For example, in *Pieris napi*, the green-veined white butterfly, the amount of apyrene sperm present is related to female receptivity, with larger numbers delaying mating (Cook and Wedell, 1999; Morrow and Gage, 2000). By delaying female remating, males are able to reduce the risk of sperm competition. Males have been shown to evolve increased production of fertile and non-fertile sperm in *P. interpunctella* when males face increased levels of sperm competition, suggesting that both types of sperm play important roles (Ingleby *et al.*, 2010).

In the current study we first aimed to investigate whether thiotepa is a reliable method to determine paternity in sperm competition and to obtain the optimal dosage for sterilising male *P. interpunctella*, as previously shown in a closely related moth *E. kuehniella* (Xu and Wang, 2010a). By using experimental evolution we examined how selection acts on traits associated with sperm defence (P_1) and offence (P_2).

Populations of *P. interpunctella* have been evolving under male and female biased adult mating regimes for around 90- 96 generations. Males from the female biased (FB) treatment face on average no sperm competition as females only mate once, whereas the sperm of males from the male biased (MB) treatment are usually competing with rival males sperm (Ingleby *et al.*, 2010). Males evolving under these treatments have diverged in the amount of fertile and non-fertile sperm produced and in their sperm allocation patterns (Ingleby *et al.*, 2010). However, it is not known of this translates to differing sperm competitiveness and if so, how these different strategies ultimately affect male reproductive success. We predict that selection will favour traits that increase paternity under sperm competition in the MB treatment,

and males displaying these traits should have increased reproductive success. It is likely that these traits will be associated with sperm offence ability, as P_2 in insects is often under stronger selection due to the phenomena of second male precedence in a number of species (Simmons, 2001). We investigated this by allowing males to copulate with females in both first and second male roles within their selection regime, with competitors of opposing sterilisation treatment, and determining paternity by observing hatch rate of eggs. Our experimental design also allowed us to test if male ability to suppress female remating and ability to seduce a previously mated female influenced sperm competitiveness and was affected by evolutionary history. By assigning offspring to each experimental male when in first or second male roles, we also examined how variation in sperm competitiveness affects male reproductive success within and between treatments.

3.3 Methods

3.3.1 Animal husbandry

See Chapter 2; section 2.3.1.

3.3.2 Optimal thiotepa concentration

Moths from the stock population were used to determine an optimal dosage of thiotepa for sterilization. Thiotepa (N,N'N''-triethylenethiophosphoramidate; Chemical formula: $C_6H_{12}N_3PS$; Sigma–Aldrich, Co., USA) is toxic to humans (van Maanen *et al.*, 2000) and so all usage was performed under a fume cupboard. Thiotepa inhibits DNA synthesis and cell division by forming cross-links with adenine or guanine (van Maanen *et al.*, 2000), allowing males to still produce sperm, but eggs fertilised by the sperm of

thiotepa treated males fail to hatch (Nabi and Harrison, 1984; Xu and Wang, 2010a). Three concentrations of thiotepa (5%, 1% and 0.5% aqueous solution) (Xu & Wang, 2010a) and a control of distilled water were used to treat male *P. interpunctella* adults. Male moths were kept on ice before having their heads dipped into the thiotepa solution or distilled water for 10s in order to ensure ingestion of the sterilising chemical (Xu & Wang, 2010a). Males were left for 24hrs before being paired with females. Males and females were housed together in 30ml Sterlin vials from the start of the scotophase for 24hrs to allow copulation to occur. After 24hrs males were removed and vials became female egg laying vials. To determine whether sterilization persisted after the first copulation, males were immediately introduced to a second female's vial and again housed together for 24hrs before removing the male.

Females were then housed individually in egg laying vials for the duration of their life. Egg laying vials were changed every 48hrs and the fecundity (number of eggs laid) recorded. Eggs were placed in Petri dishes on double sided tape, which trapped any hatched larvae and stopped any egg cannibalism. After 48hrs the fertility of the eggs was determined. Unfertilized eggs become shrivelled and yellow, whereas fertilized eggs remain white, opaque and not shrivelled (Xu & Wang, 2010a). Egg hatch rate was then recorded by counting the number of larvae after a further 48hrs (4 days in total), with hatching occurring after 3 days. The fecundity (eggs laid), fertility (fertilized eggs laid) and hatch rates were recorded using a microscope. After female death copulation success was determined by dissecting the copulatory bursa and counting the number of spermatophores under a microscope. This allowed us to verify whether copulations were successful and that only one had occurred.

3.3.3 Sperm competition and remating

Experimental evolution populations of *P. interpunctella* with differing adult sex ratios were established from the stock population (see Chapter 2). In this experiment the six diverged populations were used, three each from the male biased (MB) and the female biased (FB) treatments. An experiment to determine the sperm competitiveness (P_1 and P_2), male reproductive success, as well as the ability of males to seduce non-virgin females and suppress female remating were performed after 90-96 generations of experimental evolution.

Non-virgin males were used to remove any potential confounding factors associated with P_1 and P_2 from a virgin copulation and a non-virgin copulation. Non-virgin males were gained by introducing them to a large cylinder containing an excess of females, copulations were observed and copulating pairs removed and housed in 30ml vials until copulation finished and then separated. Males were then sterilized using 1% thiopepa solution or mock sterilized using distilled water (as above), and allowed 24hrs to produce a new spermatophore. Females and competitor males were taken from the same replicate sex biased population in order to represent the conditions that moths have evolved in. Competitor males were of opposite sterilized/mock sterilized treatment group.

To determine both P_1 (proportion offspring sired when in first male role) and P_2 (proportion offspring sired when in second male role) from the same individual, males were allowed opportunities to copulate with two females, once in the first male role (P_1) and once in the second male role (P_2). Virgin females were paired randomly with non-virgin focal sterilized/mock sterilized males and copulation was allowed to occur over a 4hr period from the start of scotophase, with copulation events recorded every 15mins, *P. interpunctella* copulations last approximately 45mins (personal

observation). Focal males were then removed from mating vials which became female egg laying vials. The following day at the start of scotophase non-virgin females were introduced to focal males' vials and females previously mated to focal males were introduced to the vials of twice mated competitor males. Copulations were allowed to occur within 4hrs and were recorded every 15mins. If second copulations occurred, females were then housed in egg laying vials individually until death and males were frozen for later body size measurements. Female egg laying vials were changed every 48 hrs with fecundity, fertility and hatch rate recorded as in the optimal thiotepa concentration experiment (see above). If no second copulation occurred females and males were separated and further copulation attempts were allowed the following day and a further two days or until a second copulation occurred. If males failed to remate they were frozen for body size measures. All eggs laid before a females' second copulation were counted from female egg laying vials and if females didn't remate eggs were counted every 48hrs until death.

Paternity was assigned by the proportion of eggs hatched, with non-hatching fertilized eggs (remain opaque white and not shrivelled in appearance) assigned those sired by the sterilized male and successful hatching assigned to the mock sterilized male. Hatch rates were corrected by the average proportion of fertilized eggs laid that didn't hatch when females were mated with mock sterilized males and also by the average proportion of fertilized eggs which did hatch when mated with a sterilized male. These average proportions were calculated from the optimal dosage thiotepa experiment (see above).

3.3.4 Male reproductive success

The number of offspring sired by males was determined by combining the total offspring sired from focal males' first (P_1) and second (P_2) copulations. Offspring sired from the males' first copulation was defined as the number of eggs a female laid before a second copulation (corrected by average proportion eggs laid which were fertile and the number of fertile eggs which did not hatch, determined from both optimal thiotepa concentration and sperm competition experiments) and the offspring assigned after a second copulation occurred. Offspring sired from the males second copulation are eggs sired from his second copulation only. Total offspring sired is defined as the combined offspring from males' first and second copulations, including assigned eggs when males and females didn't remate.

3.3.5 Statistical analysis

All data analyses were carried out using R statistical software version 3.1.2 (R Core Team, Vienna, Austria). Minimal adequate models were reached by stepwise removal of non-significant terms and models checked for homoscedasticity of residuals.

Generalized Linear Models (GLM's) with quasi-Poisson error structure to account for over-dispersion were used to analyse fecundity, fertility and hatch rate of females used in the optimal thiotepa dosage experiment. Post hoc Tukey's honest significance difference tests were then performed to see which treatments were significantly different.

To test for differences in sperm competitiveness between sex ratio treatments, GLM's with quasi-binomial error structure were used, with P_1 and P_2 as response variables and sex ratio treatment, fecundity before second copulation and male

relative body size (focal male size – competitor male size) as explanatory variables.

Mixed effect models were not used due to over-dispersion, and so variation between replicates cannot be accounted for. To observe any potential replicate effects separate GLM's was performed for each of the two sex ratio treatments, with quasi-binomial error structure, with P_1 and P_2 as response variables and replicate (instead of sex ratio treatment), fecundity before second copulation and male relative body size as explanatory variables, together with post hoc tests. Quasi-binomial error structure was used instead of transformed proportions with a normal error distribution, as this allowed for weighted analyses based on observed sample sizes with large variation. To explore if P_1 and P_2 were correlated analysis was restricted to males with both measures using GLM's with quasi-binomial error structures as the model above, but with P_1 and P_2 as added explanatory variables in models analysing P_2 and P_1 , respectively.

To test for differences in male and female remating rate between sex ratio treatments a GLM with quasi-binomial error structure, with male and female remating (yes or no) as the response variable and sex ratio treatment as the explanatory variable. A relationship between a male's ability to seduce a non-virgin female and sperm defence (P_1) ability was assessed using a GLM with quasi-binomial error structure with P_1 as the response variable and sex ratio treatment and male remating success as explanatory variables.

Relationships between sperm competitiveness and male reproductive success was analysed using GLM's with quasi-binomial error structure, with P_1 and P_2 as response variables and sex ratio treatment and male reproductive success (eggs sired from first and second copulations, respectively) as explanatory variables. To assess for any relationships between male and female remating probability (yes, no) and male

reproductive success (total eggs sired) a GLM with quasi-poisson error structure was used, with total offspring sired as the response variable and observed mating system (polygyny (male remated, females mated once), polyandry (female remated, male mated once) or promiscuous (male and female remated)) and sex ratio treatment as explanatory variables. Finally, differences between sex ratio treatments and male reproductive success was analysed using a GLM with quasi-poisson error structure with total offspring sired as the response variable and sex ratio treatment as the explanatory variable.

3.4 Results

3.4.1 Optimal thiotepa concentration for male sterilization

The effect of thiotepa treatment on *P. interpunctella* males' mating success and the fecundity, fertility and hatch rate of those females mated to treated and untreated males are summarized in Table 3.1. The majority of males from 0.5% and 1% thiotepa treatments achieved successful copulation and insemination of females, with the greatest numbers of failures observed in males treated with 5% thiotepa. Female fecundity was influenced by thiotepa treatment (GLM: $F_{6,95} = 2.91$, $P = 0.012$), however it was only females mated with twice mated 5% thiotepa treated males that differed significantly (post hoc Tukey's honest significance difference test: $P = 0.042$). Overall, thiotepa treatment had no effect on the number of fertile eggs a female laid (GLM: $F_{6,95} = 1.89$, $P = 0.090$). In contrast, there was a highly significant effect of thiotepa treatment on their hatch rates (GLM: $F_{6,94} = 56.67$, $P < 0.001$); those females copulated with 1% and 5% treated males had hatching success rates close to zero. Taking into consideration all aspects of reproduction (Table 4.1) using 1% thiotepa concentrations

is the most appropriate concentration for determining sperm competitiveness in *P. interpunctella*, because hatch rates are close to zero but the majority of males successfully copulated and inseminated females.

Table 3.1. Effect of thiotepa concentration on *P. interpunctella* male mating success and female fecundity, fertility and hatch rates \pm SE.

Treatment	<i>n</i>	death within 24hr	Males		Females		
			% successful copulations	% achieving inseminations*	Fecundity	fertility	hatch rate
5% thiotepa (1)	20	1	74	71	71.2 \pm 16.3 a	68.0 \pm 17.0 a	0.7 \pm 0.6 a
5% thiotepa (2)	14	n/a	71	50	81.6 \pm 4.3 b	74.0 \pm 4.1 a	0.3 \pm 0.2 a
1 % thiotepa (1)	23	0	85	88	102.9 \pm 15.2 a	100.1 \pm 15.3 a	3.1 \pm 1.4 a
1 % thiotepa (2)	17	n/a	94	94	107.8 \pm 16.0 a	103.3 \pm 15.9 a	0.3 \pm 0.2 a
0.5% thiotepa (1)	25	0	87	95	109.5 \pm 13.2 a	102.0 \pm 13.2 a	51.0 \pm 9.1 b
0.5% thiotepa (2)	20	n/a	65	100	64.8 \pm 13.1 a	60.8 \pm 12.9 a	35.5 \pm 8.7 b
control	15	0	100	93	120 \pm 8.2 a	93 \pm 12.1 a	91.6 \pm 1.7 c

- (1) and (2) in treatment correspond to males' first or second copulation, respectively

- *proportion of mated females that laid fertile eggs

- different letters represent significant differences (post hoc Tukey's honest significance difference test $P < 0.05$)

3.4.2 Sperm competition

By mating females with both 1% thiotepa treated and mock sterilized males, the sperm competitive ability of 238 focal males was quantified (139 from the MB treatment and 99 from the FB treatment). From these both P_1 and P_2 competitiveness was determined from 137 males (89 from the MB treatment and 48 from the FB treatment). There was large variation in P_1 and P_2 , ranging from 0 to 100% in both MB and FB treatments. However, there was no difference in male sperm competitiveness between sterilized and mock sterilized males when 1% thiotepa treated males mated

in the first, P_1 (GLM: $F_{1,190} = 0.81, P = 0.371$) or second, P_2 (GLM: $F_{1,181} = 0.04, P = 0.835$) roles.

Overall male sperm defence (P_1) and offence (P_2) ability significantly differed between treatments (Table 3.2; Figure 3.1). On average, P_1 was higher in the MB treatment and P_2 higher in the FB treatment. P_1 and P_2 significantly differed between replicates (GLM: $P_1: F_{5,186} = 2.85, P = 0.017$; $P_2: F_{5,177} = 2.64, P = 0.025$). However, following post hoc analysis only two replicates significantly differed from each other (MB2 – MB3: $P_1: P = 0.007$; $P_2: P = 0.005$, appendix Figure 3.1A). The number of eggs laid by females before their second copulation, was not related to sperm competitiveness (GLM: $P_1: F_{1,189} = 1.49, P = 0.224$; $P_2: F_{1,180} = 0.44, P = 0.508$; Table 3.2). Male relative body size had no significant effect on sperm competitiveness (GLM: $P_1: F_{1,188} = 0.10, P = 0.746$; $P_2: F_{1,179} = 0.43, P = 0.836$; Table 3.2), although males from the MB sex ratio treatment were found to be significantly larger than males from the FB sex ratio treatment (GLM: $F_{1,236} = 9.13, P = 0.002$: FB: mean \pm SE = 3.23 ± 0.03 mm; MB: 3.40 ± 0.03 mm). There was no evidence of a relationship between sperm defence and offence ability, P_2 success did not influence P_1 (GLM: $F_{1,137} = 0.92, P = 0.338$) and P_1 success did not influence P_2 (GLM: $F_{1,137} = 2.35, P = 0.127$).

Table 3.2. Results from GLM analysis of P_1 and P_2 competitiveness. Significant P values are highlighted in bold.

Response Variables	P_1			P_2		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Sex ratio treatment	1,190	4.24	0.041	1,181	4.06	0.045
Fecundity before second copulation	1,189	1.49	0.224	1,180	0.44	0.508
Relative male body size	1,188	0.1	0.746	1,179	0.43	0.836

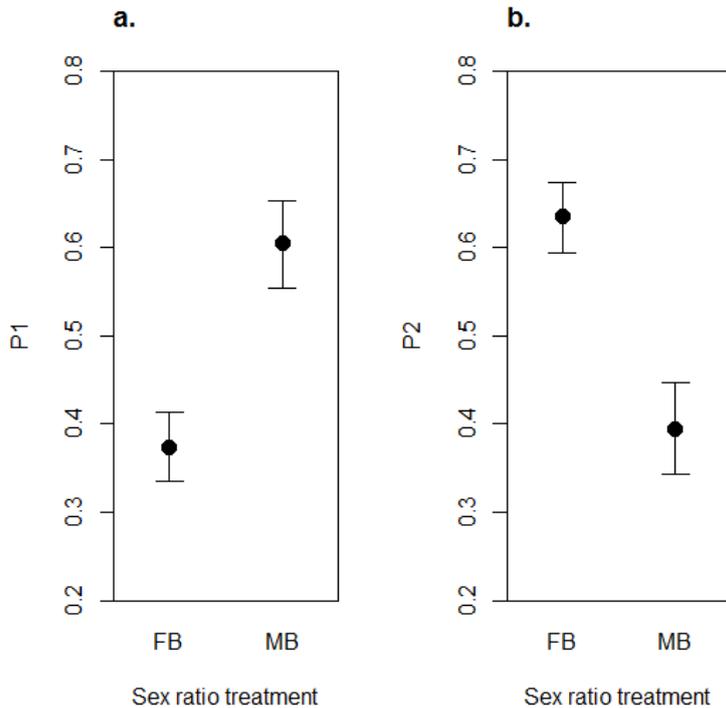


Figure 3.1: Mean sperm competitiveness in P₁ (a), and P₂ (b) roles of males from female biased (FB) or male biased (MB) populations. MB males sire higher proportion of offspring in the P₁ role, whilst FB males sire more in the P₂ role. Error bars indicate ± 1SE.

3.4.3 Male seduction and suppression ability

The proportion of females that remated significantly differed between treatments (GLM: $F_{1,229} = 5.35$, $P = 0.022$; Figure 3.2), with 89.1% of females remating in the MB treatment compared to 77.7% from the FB treatment. There was no difference in the proportion of males that remated between sex ratio treatments (GLM: $F_{1,230} = 3.21$, $P = 0.074$; Figure 3.2). The most common mating pattern in both sex ratio treatments observed (FB – 52.7%; MB – 73.9%) was males successfully seducing a second female (non-virgin) and failing to suppress the first female mated with from remating, hereafter ‘promiscuous’ mating system. Monogamous males (i.e. mated once) and polyandrous females (i.e. remated) were observed in both sex ratio

treatments (FB – 25.3%; MB – 15.7%), hereafter ‘polyandrous’ mating system.

Remating males paired with monandrous females (i.e. female only mated once) were also observed (FB – 22%; MB – 10.4%), hereafter ‘polygynous’ mating system. No monogamous pairs (i.e. males and females only mating once) were observed.

Males which successfully seduced a non-virgin female had significantly higher sperm defence (P_1) ability compared to those males that failed to seduce a non-virgin female (GLM: $F_{1,190}=10.71$, $P = 0.001$; Figure 3.3), there was no interaction existing with sex ratio treatment (GLM: $F_{1,188}=2.48$, $P = 0.117$).

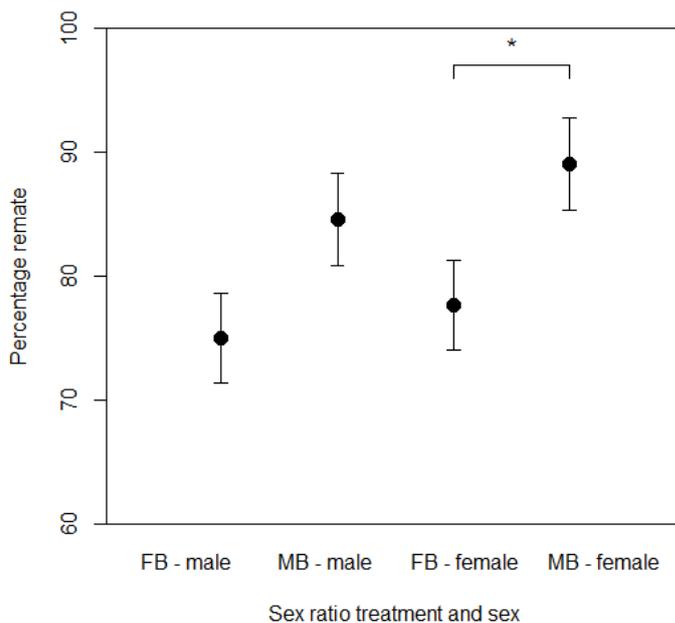


Figure 3.2: Mean percentage of remating male and female moths in male biased (MB) and female biased treatments. Females in the MB remated significantly more than females from the FB (* $P < 0.05$). Error bars indicate $\pm 1SE$.

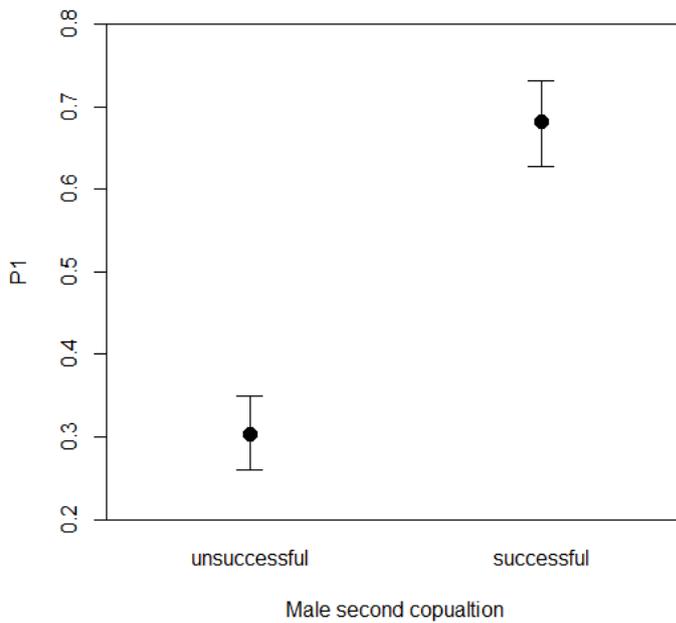


Figure 3.3: Mean sperm defence of males (P_1 – proportion sired by the first of two males) combined from both sex-biased treatments that failed to seduce (unsuccessful), or did seduce (successful) a non-virgin female to remate. Error bars $\pm 1SE$.

3.4.4 Male reproductive success

There was a significant interaction between the number of offspring sired by males in the first (P_1) role (fecundity before second copulation and offspring sired in sperm competition following second mating) and sex ratio treatment influencing males' P_1 sperm competitive success (GLM: $F_{1,189} = 6.62$, $P = 0.011$; Figure 3.4a). In both FB and MB treatments, as male P_1 sperm competitiveness increased so did the number of offspring sired. However, there is a significant difference in slopes between treatments (Figure 3.4a). At low P_1 values FB males sired more offspring than MB males of equal P_1 success. As P_1 competitiveness increased, MB males sired greater

number of offspring than FB males when P_1 values were equally high. P_2 competitiveness also increased with the number of eggs sired from P_2 copulation (GLM: $F_{1,175} = 172.11$, $P < 0.001$; Figure 3.4b). However, in contrast there was no significant interaction (GLM: $F_{1,173} = 0.184$, $P = 0.668$) and there was no significant difference between sex ratio treatments, although a trend for MB males to sire more offspring as P_2 competitiveness increased (GLM: $F_{1,174} = 3.62$, $P = 0.059$).

Male lifetime reproductive success, the total number of offspring sired from both P_1 and P_2 copulations, was influenced by males' ability to suppress female remating and ability to seduce a previously mated female (GLM: $F_{2,222} = 35.66$, $P < 0.001$; Figure 3.5), no differences between sex ratio treatment were observed (GLM: $F_{2,219} = 0.31$, $P = 0.734$). Males from polygynous mating system (male remating) sired the greatest number of offspring (150.9 ± 13.6), with males from promiscuous mating systems (both male and female remating) siring an intermediate number (87.2 ± 4.9), and males from polyandrous mating systems (female remating) siring the least number of offspring (36.6 ± 5.9) (Figure 3.5).

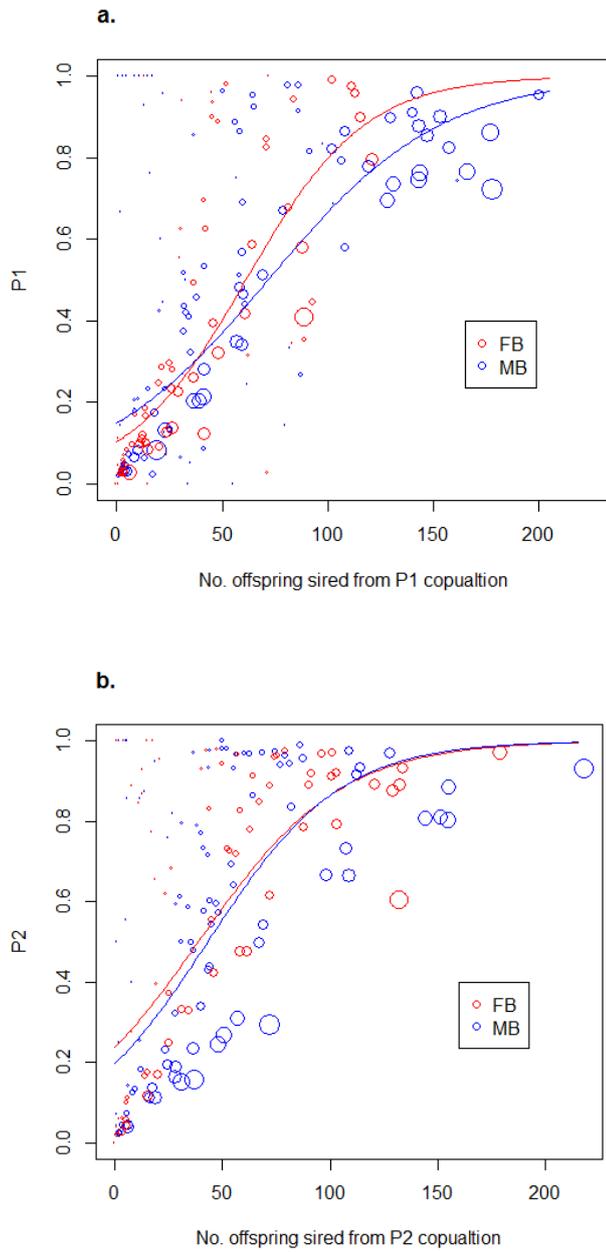


Figure 3.4: Scatterplots showing the number of offspring sired from P_1 (a) and P_2 (b) copulations by focal males from the female biased (FB; red dots) and male biased (MB; blue dots) treatment. The diameter of each point is proportional to the number of eggs a female laid after the second copulation.

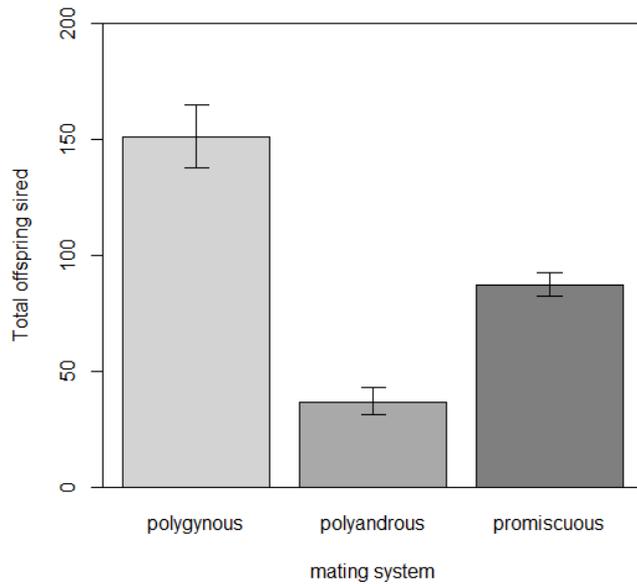


Figure 3.5: Mean number of offspring sired by males from different observed mating systems (polygynous: male remate, female mates once; polyandrous: male mates once, female remates; promiscuous: both male and female remate. Error bars \pm 1SE.

Despite differences in average sperm competitiveness (P_1 and P_2 ; Figure 3.1), the observed number of offspring sired from P_1 copulations (Figure 3.4), the proportion of females that remated (Figure 3.2) between sex ratio treatments, and differences in the total number of offspring sired from the different mating systems (Figure 3.5), overall, we observed no differences in the total number of offspring sired by males between sex ratio treatments (GLM: $F_{1,223} = 1.01$, $P = 0.315$; Figure 3.6).

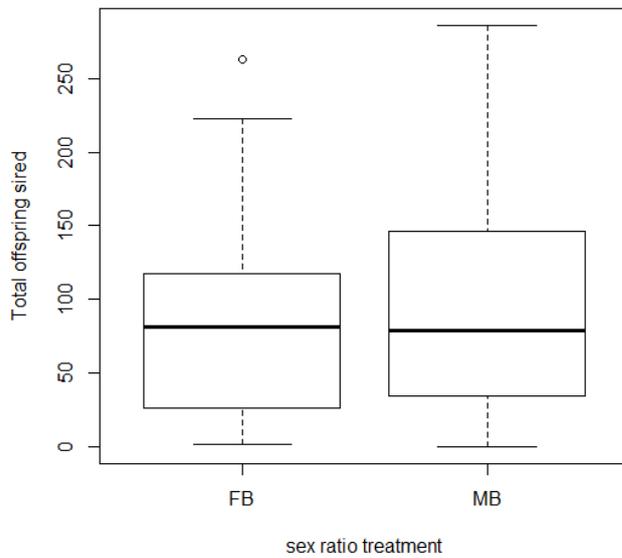


Figure 3.6: Boxplot showing median total of offspring sired by males from both the female biased (FB) and male biased (MB) populations. Inter-quartile ranges also shown.

3.5 Discussion

This study shows that sperm defence (average P_1) and sperm offence (average P_2) ability was significantly different between the two adult sex ratio treatments. Males from the female biased treatment had an increased sperm offence ability compared to males from the male biased treatment. We found no evidence of a relationship between P_1 and P_2 , suggesting that males that excelled in sperm defence did not necessarily also perform well in sperm offence. And even though sperm competitiveness differed between the adult sex ratio treatments, overall the fitness outcome for males was the same, as similar numbers of offspring were sired.

3.5.1 Sperm competition

Altering the adult mating sex ratio for over 90 generations has forced males to evolve under differing risks and intensities of sperm competition, with males in the male biased treatment experiencing higher sperm competition as females on average mate >2 , promoting increased ejaculate investment (Ingleby *et al.*, 2010). Males evolving in the FB treatment appear to be selected to protect their investment, as the majority of females do not remate in this treatment (Ingleby *et al.*, 2010). Males from the female biased (FB) treatment were found to have higher P_2 than P_1 values, indicating a 2nd male sperm precedence, as seen in a number of insects (Simmons, 2001). In contrast, P_1 was found to be higher on average in males from the male biased (MB) treatment, suggesting that males were selected to increase their sperm defence abilities and withstand competition from rival males' ejaculates. Ingleby *et al.* (2010) showed that males in the MB treatments consistently produce and donate much larger ejaculates than FB males. This may increase P_1 success through a fair raffle mechanism of sperm competition by filling and keeping the females' spermatheca full before they remate (Silberglied *et al.*, 1984; Wedell, 2005). The observed differences between sex ratio treatments indicate evolved responses of traits associated with sperm defence to increased sperm competition, suggesting adaptations that protect male *P. interpunctella* ejaculates from pre-emption are favoured when sperm competition is high.

There was no evidence of a positive relationship or trade-off between P_1 and P_2 ability within sex ratio treatments, indicating that sperm defence and offence traits evolve independently in *P. interpunctella*. Previous studies in *D. melanogaster* corroborate our findings of no correlation between P_1 and P_2 ability (Clarke *et al.*, 2005; Fricke *et al.*, 2010). However, positive relationships have been described in the

dung beetle *Onthophagus taurus* (House and Simmons, 2006), as well as trade-offs in the red flour beetle *Tribolium castaneum*, where the sons of polyandrous females have increased P_2 but reduced P_1 values compared to sons of monandrous females (Bernasconi and Keller, 2001).

The lack of a relationship between P_1 and P_2 values suggests that sperm mixing is not random in *P. interpunctella* (Cook *et al.*, 1997). In the FB treatment non-random sperm mixing may be due to the second male displacing the first male's spermatophore, and inhibiting the first male's sperm migrating to the spermatheca, as seen in the moth *E. kuehniella* (Xu and Wang, 2010b). Females may also exert choice over male ejaculates, by either controlling sperm movement from the bursa to spermatheca as suggested by Cook *et al.* (1997), or by ejecting the sperm of the first male from the spermatheca (Xu and Wang, 2010b). However, spermatophore displacement cannot explain the patterns found in the MB treatment, nor females ejecting the sperm of the first male. Females may not actively bias the paternity against the second males ejaculate due to costs associated with remating (Daly, 1978); unless females gain indirect fitness benefits (Simmons, 2005). Females may gain indirect benefits from genetic variation of offspring through remating (Tregenza and Wedell, 2000), or through ensuring that the first males sperm is competitive and will produce sons with 'sexy sperm' (Keller and Reeve, 1995). Females may preferentially allocate the sperm of first males as these males mated faster in the population, suggesting that they are the most attractive and/ or more competitive males that may go on to produce 'sexy sons' (Fisher, 1930; Kirkpatrick, 1982; Hosken *et al.*, 2008). While these mechanisms are difficult to observe or test empirically (Birkhead, 1998; Pitnick and Brown, 2000), cryptic female choice is recognised as a powerful force in

sexual selection, and it is clear females are not passive in post-copulatory selection (reviewed in Eberhard, 1996).

3.5.2 Male seduction and suppression ability

Across both male and female biased sex ratio treatments, a male's ability to seduce a non-virgin female was shown to be associated with P_1 sperm competitiveness. Males that were successful at mating with a non-virgin female had significantly higher sperm defence ability, compared to males who failed to remate again. This corroborates previous research in *P. interpunctella*, where males that mated once were likely to seduce another female to mate, as well as increase their paternity share through donation of greater numbers of sperm (Lewis *et al.*, 2013). Male ability to seduce non-virgin females is likely to be correlated with overall male attractiveness (Hosken *et al.*, 2008). This suggests that attractive males are superior P_1 sperm competitors, or that females preferentially use the sperm of 'attractive males', as seen in the spider *Physocyclus globosus* for example (Peretti and Eberhard, 2010). Male attractiveness is related to sperm competitiveness in a number of species, such as *T. castaneum* (Lewis *et al.*, 1994), the guppy *Poecilia reticulata* (Evans *et al.*, 2003) and the fruit fly *Drosophila simulans* (Hosken *et al.*, 2008), although trade-offs have also been found in the fire fly *Photinus greeni* (Demary and Lewis, 2007). The mechanism for the observed high P_1 values of successful *P. interpunctella* males in seducing non-virgin females is unknown; it is not clear whether it is due to male sperm competitiveness, females biasing paternity (Cook *et al.*, 1997), or both.

The most commonly observed pattern of mating in this study was for both males and females to remate i.e. a promiscuous system. This does not reflect the mating patterns of the conditions moths evolved in, but a consequence of our staged

matings. However, significantly fewer females remated in the FB treatment compared to the MB treatment. It is unclear if the observed result is because males from the FB treatment have an increased ability to suppress female remating, or due to the evolved differences of the females, as they evolved to only mate once on average in the FB treatment (Ingleby *et al.*, 2010).

3.5.3 Male Reproductive Success

An interaction between sex ratio treatments and numbers of eggs sired from a male's first copulation was found to be associated with P_1 sperm competitiveness. Males from the MB treatment sire more offspring when excelling in sperm defence, yet fewer when compared to males of equal P_1 competitiveness from the FB treatment. When of equal P_2 competitive ability, males from the FB consistently sire more offspring compared to males from the MB treatment. Males in the MB treatment have evolved in populations where females are limited, and so virgin females are rare and sperm competition high. Previous research has shown *P. interpunctella* to allocate sperm according to female mating status (Cook and Gage, 1995), and as such may increase investment in virgin females as they represent a greater pay-off, especially if males can also suppress female remating. A combination of both increased male investment, and potential paternity biasing by the female (discussed above), may contribute to the observed high P_1 offspring numbers sired by males in the MB treatment.

Male body size differed between the sex ratio treatments, with males from the MB being significantly larger, corroborating previous findings (Ingleby *et al.*, 2010; Chapter 3). Ingleby *et al.* (2010) suggested that larger *P. interpunctella* males may gain

pre- and post-copulatory advantages. However, we previously found no effect of male body size in either FB or MB treatments in relation to male pre-copulatory mating success (Chapter 2). Body size is known to positively relate to sperm competitiveness in *D. melanogaster* (Pitnick *et al.*, 2001). However, in this study we found no evidence that relative body size influenced sperm competitiveness of males. In other species of Lepidoptera, larger body size is also shown to positively correlate with amount of ejaculate produced (Bissoondath and Wiklund, 1996; Wedell and Cook, 1999). Larger male size may be favoured in sperm competition due to males producing larger spermatophores or more sperm (Wedell, 1997).

Unsurprisingly, males sire the greatest number of offspring by seducing non-virgin females, and simultaneously suppressing female remating of his first mate. Despite the differences observed between sex ratio treatments regards sperm competitiveness and reproductive success, we found that males from male and female biased treatments sire equal numbers of offspring overall. Although differing tactics are employed by the sex ratio treatments, this results in the same overall pay-off to males in terms of their post-copulatory reproductive success.

3.5.4 Conclusion

In conclusion, our study shows that thiotepa can reliably be used for determining outcomes of sperm competition in *P. interpunctella*. When facing increased sperm competition due to female remating, males evolving under male biased treatments are selected for traits associated with increased sperm defence. However, we cannot rule out that females may be biasing paternity towards specific males. Males from the different sex ratio treatments adopt different strategies to

maximise reproductive success, but ultimately sire equal numbers of offspring. Whilst post-copulatory sexual selection is a pervasive force in nature, it appears that in *P. interpunctella* either sperm defence or offence tactics are equally important to males' overall paternity shares.

4. Evolved Divergence in Protection against a Viral Pathogen

4.1 Abstract

Immunity is costly, and is often traded-off against other key fitness traits including future reproductive episodes. Immune response can be estimated through measures of circulating phenoloxidase levels, a key component of the insect immune system. Here we used replicate populations of the Indian meal moths, *Plodia interpunctella*, that have diverged in circulating levels of phenoloxidase. We can therefore test whether these evolved differences in phenoloxidase titres confer differing protection against a viral pathogen. Third instar larvae were dosed with *P. interpunctella's* granulosis virus, PiGV. We found that a significantly higher proportion of larvae evolving under an adult female-biased sex ratio with lower levels of circulating phenoloxidase show signs of infection, compared to larvae evolving under an adult male-biased sex ratio associated with higher phenoloxidase levels. Dose treatments were also significant, with larvae showing signs of infection as viral concentration reach lethal dose. Increasing viral load leads to lower survival rates in low phenoloxidase female-biased populations. Increased courtship and mating frequencies by males in female-biased populations may instigate this trade-off, leading to increased susceptibility to viral pathogen. Altering levels of sexual selection and sexual conflict has led to divergence in evolved susceptibility to a viral pathogen.

4.2 Introduction

There are varying challenges an organism faces, and one such challenge is the pressure placed on the immune system. Immunity in both vertebrates and invertebrates stems from either an innate response, where an individual will have immunity against pathogens regardless of its environment; or an acquired response, where an immune response is only generated when the individual is under attack from pathogens (Zuk and Stoehr, 2002; Schmid-Hempel, 2003). Regulation of the immune response may be condition-dependant, with individuals raised in better conditions being able to invest more in an immune response than those exposed to poorer conditions (Lazzaro and Little, 2009). Environmental conditions that are known to affect immune responses include temperature (e.g. Frid and Myers, 2002; Karl *et al.*, 2011), diet quality (e.g. Moret and Schmid-Hempel, 2000; Fellous and Lazzaro, 2010), and population density (e.g. Barnes and Siva-Jothy, 2000; Wilson *et al.*, 2002). These factors will fluctuate in the environment, and are likely to influence immune system responses to pathogen attack. Evidence also suggests that diet, temperature and population density can act synergistically, particularly in wild populations (Triggs and Knell, 2012), causing greater demands on the immune system.

According to life-history theory, there may be flexibility in the use of resources (energy) to mount an immune response. As organisms have a finite amount of resources that are also invested in somatic maintenance and reproduction, there will be limitations to the immune response an individual can generate (Sheldon and Verhurd, 1996). Trade-offs between reproduction and immunity is common in both vertebrates (reviewed in Martin *et al.*, 2008) and insects (e.g. Rolff and Siva-Jothy, 2002; reviewed in Lawniczak *et al.*, 2007). For example, reproductive success in several

species of mosquitoes is shown to decrease when resistance to varying pathogens is increased (Ferdig *et al.*, 1993; Ahmed *et al.*, 2002). Immune response trade-offs may also be sex-specific, with a “live fast, die young” approach being favoured in males (Trivers, 1972; Rolff, 2002). Sexes gain fitness benefits through different reproductive routes, typically with high mating frequencies being favoured in males, whilst females increase their success through higher lifetime fecundity (Arnqvist and Nilsson, 2000). Immunity will be up- or down-regulated according to the different energetic demands of the sexes. For example, age-dependant trade-offs between reproduction and immunity is present in male cotton bollworm, *Helicovera armigera*, but not in females (McNamara *et al.*, 2013a).

Demands for reproductive investment are dependent on differential selection pressures, but these pressures are often difficult to quantify accurately. Experimental evolution is a powerful way to experimentally vary the pressures placed on the sexes, and this allows us to examine specific changes in reproductive investment over relatively short time frames. For example, research shows that after being exposed to many generations of increased risk of sperm competition, male yellow dung flies evolve larger testes size than males from populations evolving without sperm competition (Hosken and Ward, 2001). Manipulating the adult mating sex ratio has created differences in selective pressure in several species with concurrent responses by evolving populations (Wigby and Chapman, 2004; Leftwich *et al.*, 2012). Biasing the adult sex ratio causes varying levels of sexual selection and sexual conflict to be faced by the sexes. Selection is expected to be stronger on the more numerous sex, as there is increased intra-sexual competition to secure matings (Weir *et al.*, 2011). However, as one sex gains fitness advantages, it may result in sexual conflict constraining the other sex in reaching its fitness optimum (Hosken and Stockley, 2005). Differing

reproductive strategies by males and females causes sexual conflict and sexual selection to vary between populations (Parker, 2006), which can lead to divergence in fitness outcomes (Leftwich *et al.*, 2012; Chapter 3).

The moth *Plodia interpunctella* is a pest of stored grains, which means laboratory conditions are very similar to its natural environment. Previous research shows that varying the adult sex ratio in evolving populations of *P. interpunctella* causes divergence in mating frequencies and level of sperm competition (Ingleby *et al.*, 2010). It has also been shown that circulating phenoloxidase (PO hereafter) have diverged with lower levels being present in female-biased *P. interpunctella* populations compared to moths evolving under a male-biased adult sex ratio (McNamara *et al.*, 2013b). In insects, PO is a key enzyme used in the encapsulation of foreign bodies (Triggs and Knell, 2012), and increased amounts of PO are shown to correlate with increased ability to resist attack of pathogens (Schmid-Hempel, 2003).

Naturally occurring pathogens of *P. interpunctella* include those from the baculoviruses family. *P. interpunctella* granulosis virus (*PiGV* hereafter) is a highly species-specific virus that only affects the larval stages (Sait *et al.*, 1998). When virus particles are ingested, *PiGV* infection begins in the midgut, spreading to the hemocoel of larvae (Saejong *et al.*, 2010). Swift replication of the virus ensures that individuals who are unsuccessful in mounting an effective immune response will show signs of infection and prevented from pupation (Seajong *et al.*, 2010). Frequently *PiGV* has been examined in terms of biological pest control (Mohandass *et al.*, 2007), in studies of host-parasite population dynamics (Sait *et al.*, 1994; Boots and Mealor, 2007) as well as insect immune defence mechanisms (Boots and Begon, 1993; Boots and Begon, 1994).

Our study involves dosing Indian meal moth (*P. interpunctella*) larvae with the moth-specific pathogen, *PiGV*. Here we used the same female biased (FB) and male biased (MB) experimental evolution populations as in McNamara *et al.* (2013b), where it was found that levels of circulating PO have diverged between the moth populations. Based on the previous findings of McNamara *et al.* (2013b) we predict that individuals from FB populations will be more susceptible to *PiGV* infection than individuals from the MB populations, as they have lower circulating PO levels. Four increasing doses of *PiGV* are used in this experiment alongside a control treatment. As viral concentrations increases, we expect a higher proportion of larvae infected with *PiGV*, eventually reaching a lethal dose. We discuss the outcomes of this experiment in the context of sexual conflict, sexual selection, and immune response, and possible trade-offs.

4.3 Methods

4.3.1 Animal Husbandry

See Chapter 2; section 2.3.1.

4.3.2 Experimental Evolution Populations

See Chapter 2; section 2.3.2.

4.3.3 Virus Preparation

Stock of the *Plodia interpunctella* granulosis virus was made using a method outlined in Smith and Crook (1988). Virus was obtained from the homogenate of infected individuals. It was then purified, and diluted with 2% sucrose and 0.1% Coomassie Brilliant Blue R Dye in distilled water. Serial dilutions of the virus were carried out. The concentrations were as follows: 2.5%, 0.25%, 0.025%, 0.0025%, and

0.00025%. These concentrations covered lethal dose values from 0 to 100 according to previous dosing carried out on *P. interpunctella* stock (Sharpe *et al.*, unpublished data). A control was used, containing sucrose water with blue dye used in virus preparation above. Serial dilutions and controls were stored in 1.5mL Eppendorf's at -20°C until ready for use.

4.3.4 Preparation and Dosing of Sex-Biased Populations

Dosing of sex-biased populations occurred after approximately 95- 100 generations of experimental evolution. Individual males and females were taken at random from each of the three replicate populations of MB and FB respectively. Male and female larvae were randomly selected and allowed to mate as adults under an equal sex ratio, so as to remove any possible maternal effects. Eggs were collected over a period of 3 days. The resulting F1 eggs were transferred to a labelled 0.5 litre stock pot with an excess of larval medium inside. Once the F1 generation had reached 5th instar, 90 males and 90 females from each population were collected and housed in single-sex pots. Upon eclosion into adult moths, they were placed into an egg collector. F2 eggs were collected every 24 hours. The resulting F2 eggs were placed into a small pot with larval medium, and monitored for growth into 3rd instar ready for dosing (~ 11- 14 days later).

Eppendorf's of the varying virus concentrations and control dose were removed from freezer and allowed to defrost. Approximately 300 third instar larvae were collected for each of the FB and MB experimental evolution lines to be used in the 5 treatment groups. 50 larvae were randomly allocated to one of the 5 doses of *PiGV*, including the control. Multiple drops of the virus solution were pipetted into the petri dish containing larvae. Paper towel soaked in water was then placed over the top

of a petri dish and the lid secured in place. Larvae were left for 30 minutes, or until larvae had consumed half of their body's length of solution (blue dye visible through body cavity wall). Individuals were then transferred into separate wells in a 25 well-plate containing larval medium. Plates were labelled according to dose received, had paper towel folded inside of lid (to minimise larval escape), secured with tape and placed inside an incubator. Incubator was maintained on the same temperature and light: dark cycle as above.

F2 larvae were checked 15 days (around fourth instar) after dosing for symptoms of infection by PiGV. *P. interpunctella* larvae that are infected appear opaque white in colour and inhibited from eclosion into adults (Seajong *et al.*, 2010), in contrast to healthy larvae who remain yellow in colour. The proportion of infected and non-infected larvae was recorded for each of the populations for each dose treatment. Total larvae numbers that were dosed with virus for all treatments, including controls were: FB1 $n = 375$; FB2 $n = 250$; FB3 $n = 127$; MB1 $n = 400$; MB2 $n = 250$; and MB3 $n = 250$.

4.3.5 Survival of Larvae

Final larvae counts were recorded when each population and dose treatment were checked for viral infection 15 days after dosing. Larvae were deemed to have suffered early death if they could not be accounted for from total numbers that were dosed for each treatment, irrespective of infection or not. Final surviving larvae numbers for all treatments including controls were: FB1 $n = 247$; FB2 $n = 128$; FB3 $n = 57$; MB1 $n = 339$; MB2 $n = 171$ and MB3 $n = 203$.

4.3.5 Statistical Analysis

A generalized linear model (GLM) with binomial error distribution was used for analysis of proportion of larvae infected, and early death of larvae. Independent variables in model were: selection, replicate, dose, and dose*selection. Analysis was conducted both with replicate as a factor and nested within selection regime, but significance was found not differ between these methods, so here we report results from the former analysis.

All statistical analysis was conducted using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

4.4 Results

4.4.1 Dose Response

Selection regime had a significant effect on proportion of larvae infected (GLM; $\chi^2_1 = 13.958$, $N = 871$, $P < 0.001$). Overall, female biased populations had higher proportions of infected larvae than male biased regimes (mean \pm 1SE; female-biased = 0.51 ± 0.060 , male-biased = 0.25 ± 0.037 ; Figure 4.1). Dose treatment significantly affected both selection regimes, with a higher proportion of larvae showing signs of infection as dose exposure increased exponentially ($\chi^2_3 = 109.032$, $N = 871$, $P < 0.001$; Figure 4.1; mean \pm 1SE Dose 1: 0.06 ± 0.017 ; Dose 2: 0.34 ± 0.046 ; Dose 3: 0.87 ± 0.100 and Dose 4: 1.00 ± 0.132). There was also a significant interaction between selection regime and dose ($\chi^2_3 = 14.025$, $N = 871$, $P = 0.003$), showing that at lower doses the female-biased populations are more susceptible to infection than the male-biased

populations (Figure 4.1). Finally, the effect of replicate on proportion of larvae infected was not significant ($Wald \chi^2_2 = 0.527, N = 871, P = 0.768$).

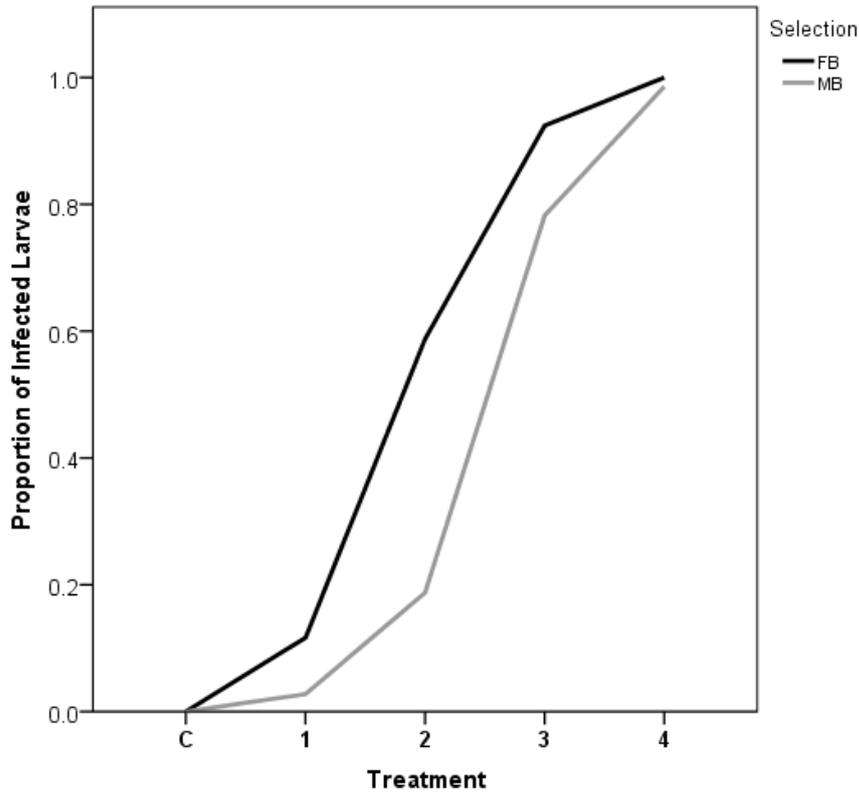


Figure 4.1: Line graph showing proportion of infected larvae for female-biased (FB; black line) and male-biased (MB; grey line) populations for each of the dose treatments. Treatments are: C = control; 1= 0.0025%; 2 = 0.025%; 3 = 0.25%; and 4= 2.5% serial dilutions of *P. interpunctella* granulosis virus (PiGV). Overall, FB populations have a higher proportion of infected larvae than MB populations.

4.4.2 Survival of Larvae

Similarly, selection regime was found to have had a significant effect on survival of larvae 15 days after dosing with virus ($\chi^2_1 = 32.211, N = 1316, P < 0.001$; Figure 4.2).

Overall, a higher proportion of larvae survived in male-biased populations irrespective of infection state compared to larvae in female-biased populations (mean \pm 1SE; female-biased = 0.45 ± 0.035 , male-biased = 0.76 ± 0.044). Dose treatment significantly affected larval survival as viral dose increased exponentially ($\chi^2_3 = 19.807$, $N = 1316$, $P < 0.001$; Figure 4.2). A lower proportion of larvae survived as viral dose concentration increased (mean \pm 1SE Dose 1: 0.73 ± 0.063 ; Dose 2: 0.70 ± 0.062 ; Dose 3: 0.53 ± 0.050 and Dose 4: 0.43 ± 0.046). This was largely driven by a lower proportion of larvae surviving overall in the female-biased populations 15 days after being inoculated with increasing viral concentrations. There was a significant interaction between selection regime and dose ($\chi^2_3 = 15.234$, $N = 1316$, $P = 0.002$; Figure 4.2), indicating that larvae from female-biased populations had proportionally fewer surviving larvae as the viral dose was increased compared to larvae from male-biased populations. Finally, there was a significant effect of replicate on proportion of larvae infected with regards to survival ($\chi^2_2 = 12.141$, $N = 1316$, $P = 0.002$). Further analysis of replicate shows significant variation within the female-biased populations ($\chi^2_2 = 9.434$, $N = 596$, $P = 0.009$) compared to the male-biased replicates ($\chi^2_2 = 5.440$, $N = 718$, $P = 0.066$).

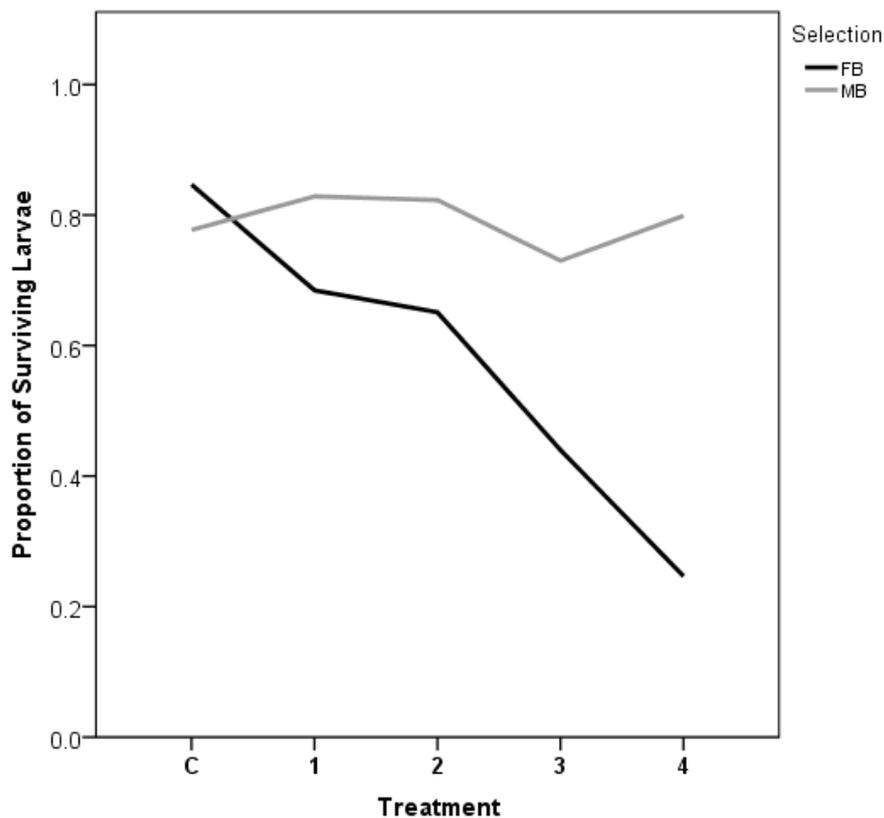


Figure 4.2: Line graph showing proportion of larvae surviving (irrespective of infection) 15 days after dosing with virus for female-biased (FB; black line) and male-biased (MB; grey line) populations for each dose treatment. Treatments are: C = control; 1 = 0.0025%; 2 = 0.025%; 3 = 0.25%; and 4 = 2.5% serial dilutions of *P. interpunctella* granulosis virus (PiGV). Overall, FB populations have fewer surviving larvae as dose increases exponentially compared to MB populations.

4.5 Discussion

Evolving under biased adult sex ratios has caused divergence in proportion of *Plodia interpunctella* infected by a viral pathogen. As predicted, larvae from female biased populations show greater susceptibility to viral infection of *Plodia interpunctella* granulosis virus (PiGV; see Figure 4.1) than larvae from the male biased populations. As viral concentrations increased, all individuals become more susceptible to infection (Figure 4.1). Regardless of infection state, we found lower survival rates 15 days after

dosing in larvae from female-biased populations than those from male biased populations (Figure 4.2).

Diverged levels of circulating phenoloxidase (PO) levels, in male biased (MB) and female biased (FB) populations has led to differing susceptibilities of larvae infected with *PiGV*. Overall, a higher proportion of larvae were infected in the FB populations when compared to the MB populations, in line with our predictions. Measure of the phenoloxidase cascade is a good indicator of the immune response generated by insects. Here we have shown that lower levels of basal PO in the FB populations (McNamara *et al.*, 2013b) leads to a higher proportion of larvae susceptible to a viral pathogen. Divergence in protection from a moth-specific pathogen is potentially due to trade-offs, according to optimal defence theory (Sheldon and Verhurst, 1996; Rigby and Jokela, 2000). With limited resources to allocate to both reproduction and an effective immune response, it has been suggested that one will be traded off against the other (Sheldon and Verhurst, 1996; Schmid-Hempel, 2003).

Evolving under a male- or a female-biased adult sex ratio has resulted in variation in mating frequencies between selection regimes. Males show variation in mating success under MB or FB populations (Ingleby *et al.*, 2010) in FB populations mate on average almost three times (2.90) over three days, compared to less than once (0.77) in the MB population (Ingleby *et al.*, 2010), with recent work showing a 17% success rate of mating when in the same competitive environmental conditions (Larner *et al.*, unpublished data). This means there is an increased expenditure on resources invested in spermatophore production and mating activity by males in the FB populations, and associated courtship costs. Virgin male *Pieris napi* butterflies that are prevented from mating for example, but still allowed to court unreceptive females

show a reduced lifespan of similar magnitude to males with access to receptive mates and allowed to both mate and court (Wedell, 2010). Similarly, male *Drosophila melanogaster* show reduced immune function when housed with four female flies, as courting and mating efforts are then increased (McKean and Nunney, 2001). In line with our findings for the FB populations, it seems that increased sexual activity in the form of increased male mating frequencies and potential associated courtship costs are traded-off against mounting better protection against a viral pathogen. This in turn may affect female immune investment. Sexually antagonistic variation in traits associated with immune response has been demonstrated in insects, such as the mealworm *Tenebrio molitor* (Rolff *et al.*, 2005). As sexes share the same genome, alleles with opposing fitness effects in the sexes may confer benefits to one sex, whilst inhibiting the other sex from reaching its optimum (Bonduriansky and Chenoweth, 2009; Van Doorn, 2009). Underlying genetic architecture can therefore play an important role in trait evolution and represent potential constraints between the sexes (Rolff *et al.*, 2005; Bonduriansky and Chenoweth, 2009). *P. interpunctella* display strong intralocus sexual conflict over shared life-history traits (Lewis *et al.*, 2011), which may potentially cause sexual dimorphism in immune response. However, due to the nature of our experimental protocol we were unable to account for the effect of sex in our analyses. Dosing at third instar did not allow for larvae to be sexed, as pigmented testes are not yet visible (Ingleby *et al.*, 2010).

Viral treatment significantly affects the proportion of larvae infected, as predicted. As viral dose increases, a higher proportion of larvae are susceptible in both MB and FB populations, likely due to the immune system being placed under higher stress due to increasing numbers of virus particles. Both male- and female-biased populations reach lethal levels of infection at 2.5% PiGV, as larvae succumb to virus

(Figure 4.1). Previous studies show that rising viral concentrations increase mortality rates in *P. interpunctella* larvae over a range of instars (Sait *et al.*, 1994). This pattern is also reflected in other Lepidopteran larvae, such as those of the Western Spruce Budworm, *Choristoneura occidentalis*, infected with a species-specific virus (Duan and Otvos, 2001). However, *P. interpunctella* moths evolving under different adult sex ratio and mating frequencies show different trajectories in relation to this lethal dose as revealed by a significant interaction between treatment and selection regime. Larvae from female biased populations are more susceptible to lower doses of PiGV than larvae from male biased populations.

As well as a higher proportion of susceptible individuals, larvae in FB populations have lower rates of survival after viral dosing. FB populations show a decreasing proportion of surviving larvae as dose concentration increases, in contrast to MB individuals (Figure 4.2). Larvae were assumed dead if they could not be found when checking for infection 15 days after dosing with PiGV. It may be argued that declines in larval survival rates in the FB populations are due to larvae escaping. However, if larval escape were to drive these observed patterns, then we would expect to see similar rates of decline across all populations. The most parsimonious explanation for the lower larval survival in female-biased populations is due to increased viral pathogenicity. This further corroborates previous work, as circulating PO is much lower in these FB populations compared to MB ones (McNamara *et al.*, 2013b) that leaves larvae prone to attack from pathogens.

Here we make use of replicate populations of moths that have diverged in circulating levels of PO (McNamara *et al.*, 2013b). We can therefore test whether these evolved differences in PO levels confer differing protection against a viral pathogen. We show that larvae from FB populations are more susceptible to PiGV than larvae

from MB populations. Immune response in insects can be quantified in a number of ways, such as haemocyte counts (e.g. Rolff and Siva-Jothy, 2002) and metabolic activity (e.g. Ardia *et al.*, 2012). Two measures of insect immunity, lytic activity and PO cascade levels, were quantified in the sex-biased populations of *P. interpunctella* (McNamara *et al.*, 2013b). Only PO levels were found to significantly differ between FB and MB populations, potentially explaining the differences in susceptibility observed here in response to dosing larvae with PiGV. In support of our findings, PO is shown to be an important mechanism in anti-viral defence in Lepidopterans (Reeson *et al.*, 1998; Popham *et al.*, 2004; Shelby and Popham, 2006; but see Seajeng *et al.*, 2010) and *P. interpunctella* challenged with virus are shown to produce offspring with higher titres of PO compared to controls (Boots and Roberts, 2012).

In conclusion, we have shown that subjecting *P. interpunctella* to varied levels of sexual conflict and sexual selection pressures by altering adult mating sex ratio has resulted in divergence in evolved immune responses. Larvae dosed with a species-specific pathogen, PiGV, are more susceptible from the FB populations than those from the MB populations. Our results further corroborate previous findings that PO is a key component of the insect immune system, and that larval mortality increases with viral load. Trade-offs may be mediating the divergence in viral susceptibility, as males from FB invest more into mating frequencies and copulation, possibly at the expense of reduced protection from a viral pathogen. Further insight into intralocus sexual conflict and its potential to inhibit sexes from reaching their optimal immunity is an avenue for further research, as well as mechanisms behind the observed viral resistance in this moth species.

5. Fecundity Selection Reveals Differences in Egg Laying Patterns in *Plodia interpunctella*

5.1 Abstract

Lifetime fitness may be maximised through selective forces acting upon strategies of individuals. One such potent force is fecundity selection that acts on traits conferring a fecundity advantage in females. Few studies have specifically examined the effects of fecundity selection and correlated changes in life-history traits known to have a strong relationship to fitness. Here we aimed to specifically target selection on fecundity in female *Plodia interpunctella* moths over a number of generations. Two replicates of upward *Fecundity Selected* lines were maintained, alongside two *Control* lines. Two life-history traits known to correlate and trade-off with fecundity, body size and longevity, were recorded. We found that after multiple generations of selection, lifetime reproductive output was successfully increased in the *Fecundity Selected (FS)* lines compared to the *Control (C)* lines. Importantly, fecundity was increased independently of changes in body size and lifespan. Egg laying patterns may vary over lifespans, so a comparison of early fecundity was made between *Fecundity Selected* and *Control* lines. In contrast, we found females from the *Control* lines to have higher early fecundity than their *Fecundity Selected* conspecifics. Differences in reproductive output are discussed in relation to the ecology of *P. interpunctella*.

5.2 Introduction

Selection shapes strategies that maximise individual lifetime fitness, with individual variation in fitness reflected in phenotypic differences (Bonduriansky *et al.*, 2008; Steiner and Taljapurkar, 2012). Resources must be acquired and assigned toward traits that increase current and future reproduction, such as fecundity and mating success, and are often traded-off against survival (Houslay *et al.*, 2015; Pincheira-Donoso and Hunt, 2015). The sexes gain fitness benefits via different reproductive routes, as a consequence of anisogamy (Schärer *et al.*, 2012). Females are typically selected to produce large eggs that carry an increased energetic cost, compared to smaller sperm in males, (Trivers, 1972; Hayward and Gillooly, 2011). Measures of reproductive output are different between the sexes, as females are limited by the number of eggs they can produce (fecundity), whereas males typically rely on higher mating success in order to fertilize many eggs (Bateman, 1948; Trivers, 1972). Previous work shows that high fecundity is strongly correlated with female fitness in many species, including vertebrates such as the Atlantic salmon, *Salmo salar* (Einum and Fleming, 2000), and in insects such as the fruit fly, *Drosophila littoralis* (Pekkala *et al.*, 2011).

Fecundity selection increases female fitness through selection on traits that confer a fecundity advantage (Pincheira-Donoso and Hunt, 2015). Darwin (1874) first proposed the hypothesis of fecundity advantage to explain female-biased sexual size dimorphism (SSD), as larger body size in females may allow for more offspring to be produced. It was thought that strong selection for increased fecundity drives correlated changes in body size, generating the evolution of female-biased SSD. Female-biased SSD is a pattern commonly found in a majority of invertebrates, as well

as some fish and reptile species (Fairbairn, 1997). Research incorporating fecundity theory has now shifted focus to include traits that may confer high fecundity, such as large abdomen size, for example in the waterstrider *Aquaris remigis* (Preziosi *et al.*, 1996), and tail length in males of the sex-role reversed *Sygnathus* pipefish, which increases area the available to brood eggs (Winkler *et al.*, 2012). Whilst body size positively correlates with increased fecundity in insects, there are selection pressures in place which inhibits them from evolving to become even larger (Blanckenhorn, 2000). Although female-biased SSD is common, previous research in lizard populations for example suggests that the strength of fecundity selection alone is not responsible for the female-biased SSD patterns (Cox *et al.*, 2003; Pincheira-Donoso and Tregenza, 2011). Sexual selection for small male body size could also drive patterns of asymmetric sexual size dimorphisms, as well as reducing risk of cannibalism by females in several species of spiders for example (Elgar and Fahey, 1996; Fromhage and Schneider, 2005).

Female reproductive success may be promoted or constrained by other extraneous factors, not just via selection on traits that increases fecundity. Environmental conditions, such as decreasing temperatures, are known to adversely affect fecundity through limiting egg maturation rates and oviposition opportunities in the speckled wood butterfly *Pararge aegeria*, for example (Berger *et al.*, 2008). Time may be a limiting resource to females, as temperature conditions may vary throughout the day or season, limiting opportunities for egg-laying (Berger *et al.*, 2012). Nutritional resources available to adults can show a marked effect on female fecundity, with diets rich in protein leading to increased reproductive output in decorated crickets, *Gryllodes sigillatus* (Archer *et al.*, 2012; Houslay *et al.*, 2015). However, in holometabolous species such as the tropical butterfly *Bicyclus anynana* for example,

larvae who are subjected to food stress during development have reduced fecundity and correlated smaller body size as adults in comparison to larvae who were fed *ad libitum*, as they are unable to compensate larval nutrients through adult feeding (Bauerfeind and Fischer, 2005). For certain species, females are able to supplement their diet by receiving nuptial gifts from males at mating (Lewis and South, 2012). Spermatophores can lead to increased fecundity in females, such as in some bush crickets for example (Brown, 1997; Gwynne, 2001; Ortiz-Jimenez and Cueva del Castillo, 2015), and in *Pieris napi*, green-veined white butterflies (Wedell, 2006). Female multiple mating is also shown to increase reproductive output in some species, which can outweigh potential costs of mating (Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000). Direct benefits of female multiple mating can include increased fecundity and fertility of eggs, such as in the example of the tomato leafminer moth, *Tuta absoluta* (Lee *et al.*, 2014), as well as genetic benefits of polyandry, such as reducing costs of inbreeding depression by increasing genetic variation in offspring, for example in the field cricket *Gryllus bimaculatus* (Tregenza and Wedell, 2002), and the Mediterranean flour moth, *Ephestia kuehniella* (Xu and Wang, 2009).

Selective forces can vary between the sexes due to differing reproductive strategies. The underlying genetic architecture may facilitate traits that confer a fecundity increase. In female heterogametic species (female ZW, male ZZ), such as Lepidopterans and birds, the genetic architecture is especially important (Ellegren, 2011), as W-linked genes (only present in females) are thought to play an important role in female fecundity and fertility (Bachtrog *et al.*, 2011). A recent study examined the expression of genes on the W chromosome in domestic chickens in comparison to their distant ancestor, the red jungle fowl (Moghadam *et al.*, 2012). It was found that chicken breeds that are under strong female-specific selection i.e. have been bred for

increased egg laying rates, show greater expression of female-biased genes on the W chromosome (Moghadam *et al.*, 2012).

To date, few studies have examined the consequences of directly selecting for increased female fecundity. The majority of such studies have focused on domestic livestock, such as increasing egg laying rates in chickens (Kerje *et al.*, 2003) and turkeys (Nestor *et al.*, 1996). To our knowledge, only one study has specifically examined female fecundity and correlated changes in life-history traits in response to fecundity selection. Using *Drosophila melanogaster*, upward- and downward-selected fecundity lines were maintained and compared with control lines (Reeve and Fairbairn, 1999). Although fecundity was not significantly increased after 20 generations of selection, there was a trend for upward selected females to lay more eggs. Despite the lack of a significant increase in fecundity (compared to control lines), upward selected females did show significantly correlated increases in body size (Reeve and Fairbairn, 1999). Downward selected females showed significantly lower fecundity levels and smaller body sizes, suggesting that fecundity selection can drive changes in genetically correlated traits (Reeve and Fairbairn, 1999).

The Indian meal moth, *Plodia interpunctella*, is an ideal organism to study the effects of female-specific selection. Life-history traits, such as development time, longevity and body size, are known to be correlated with lifetime fecundity (Lewis *et al.*, 2011). Like most insects, Lepidoptera body size is positively correlated with fecundity (Honěk, 1993; Tammaru *et al.*, 1996). Female *P. interpunctella* frequently exhibit shorter lifespans when reproductive output is higher, due to a trade-off between fecundity and longevity (Lewis *et al.*, 2011). Resources are allocated towards the energetically high demands of egg production at the expense of somatic maintenance required for longer life in many insects (Chapman *et al.*, 1998; Partridge

et al., 2005). There are no direct fecundity benefits from multiple mating in *P. interpunctella* and one mating is enough to ensure complete fertilization of eggs produced by females (Cook, 1999). Natural conditions of *P. interpunctella* are easily replicated in the laboratory, as it is a pest of stored grains.

Here, we aimed to specifically target selection on fecundity in female *Plodia interpunctella* over 6 generations. Two replicates of upward *Fecundity Selected* lines were maintained, alongside two *Control* lines. We predicted that after multiple generations of selection, individuals from the *Fecundity Selected* lines may show increased fecundity relative to their *Control* conspecifics. We also measured two life-history traits known to correlate with fecundity, body size and longevity. In order to control for correlated selection on lifespan, linear regression was performed at each generation to ensure only high fecundity females were selected for the next generation in *Fecundity Selected* lines, whilst controlling for lifespan. Body size measures were compared at the end point of the experiment in order to compare potential correlated changes in body size between females from *Fecundity Selected* and *Control* lines. Egg laying patterns may vary over lifespans, so we also compared fecundity at three days (early fecundity), as well as lifetime fecundity.

5.3 Methods

5.3.1 Animal husbandry:

See Chapter 2; section 2.3.1.

5.3.2 Stock populations:

Stock populations have been maintained in the laboratory using individuals collect in Perth, Australia in 2001. Each generation of newly eclosed moths are transferred over to a new stock pot (1L Nalgene pot) containing an excess of larval medium (~800ml of medium). The moths are allowed to mate and lay eggs until their death. After 4- 5 weeks when moths eclose, they are again transferred to new medium, thus repeating the cycle for the next generation. Prior to transferring the adult moths, stock pots were placed in a fridge at 4°C for 30 minutes to ensure moths had cooled down sufficiently to be less mobile.

5.3.3 Selection lines

Stock populations of *Plodia interpunctella* were used to set up both the *Control* and *Fecundity Selected* lines. Around 200 male and female 5th instar larvae were collected at random from the stock population maintained in the laboratory, derived from wild caught individuals established in 2001. The pigmented testes of male larvae are visible through their body cavity wall, allowing for larvae to be sexed at 4th- 5th instar (Ingleby *et al.*, 2010). Larvae were housed in single sex 0.5L Nalgene™ tubs with an excess of larval medium until eclosion. Upon eclosion the moths were placed into egg collectors, where they were able to mate for 3 days. Eggs were deposited into a conical flask. After the 72 hour mating period, eggs were transferred into new larval medium to develop for the first generation of this experiment.

Once developing larvae reached 5th instar, around 100 individuals of each sex were randomly collected and housed in single sex tubs with an excess of larval medium. Upon eclosion, adults were randomly allocated into either the *Fecundity Selected* (*FS*), or the *Control* (*C*) selection lines. 50 focal females were used for each

selection lines at each generation onwards. Two replicates each of both selection (FS) and control (C) lines were maintained for six generations.

5.3.3.i *Fecundity Selected (FS) lines*

For the *FS* lines, each focal female was housed individually in a 30mL Sterilin vial from day of eclosion, and presented with a randomly selected virgin male to mate with. Females were mated with males from within the *Fecundity Selected* lines in order to prevent diluting the effect of selection because of a lack of recombination in female Lepidopterans (Robinson, 1971). After 24 hours, the male was then removed and discarded. A modified egg collector was placed in the bottom of the vial for the females to lay eggs into. Eggs were first counted at 72 hours post-eclosion, then every 48 hours afterwards for the duration of the focal female's life in order to record total lifetime fecundity. Egg collectors were changed at every egg count. All eggs were counted, recorded and transferred into a vial labelled according to focal female. Vials contained 20mL larval medium and were topped up *ad libitum*.

Lifespan was measured as number of days from eclosion as adult moth until death. Once all focal females had died, we conducted a linear regression on lifetime fecundity and lifespan at each generation. To select the top 15 most fecund females (*Yes* treatment) for the *FS* lines, fecundity counts were used as a dependant variable whilst accounting for the independent effect of lifespan, by regressing fecundity on lifespan and saving the standardized residuals. The residuals allowed us to select those females who had the highest fecundity whilst controlling for lifespan. This is because of a trade-off between lifetime fecundity and longevity, and we did not want to inadvertently select on lifespan *per se*. The eggs collected from these 15 *Yes* treatment

females were combined together in a 1L Nalgene™ stock pot to provide moths for the next generation (see Figure 6.1). The remaining 35 female's eggs were pooled together for the *No* treatment, in order to be used as mating partners in next generation (see Figure 6.1). Once all focal females had died, they were frozen at -20°C in order to measure body size. Body size (mm) was estimated using a measure of the right wing length according to the methods described in Reid (1976).

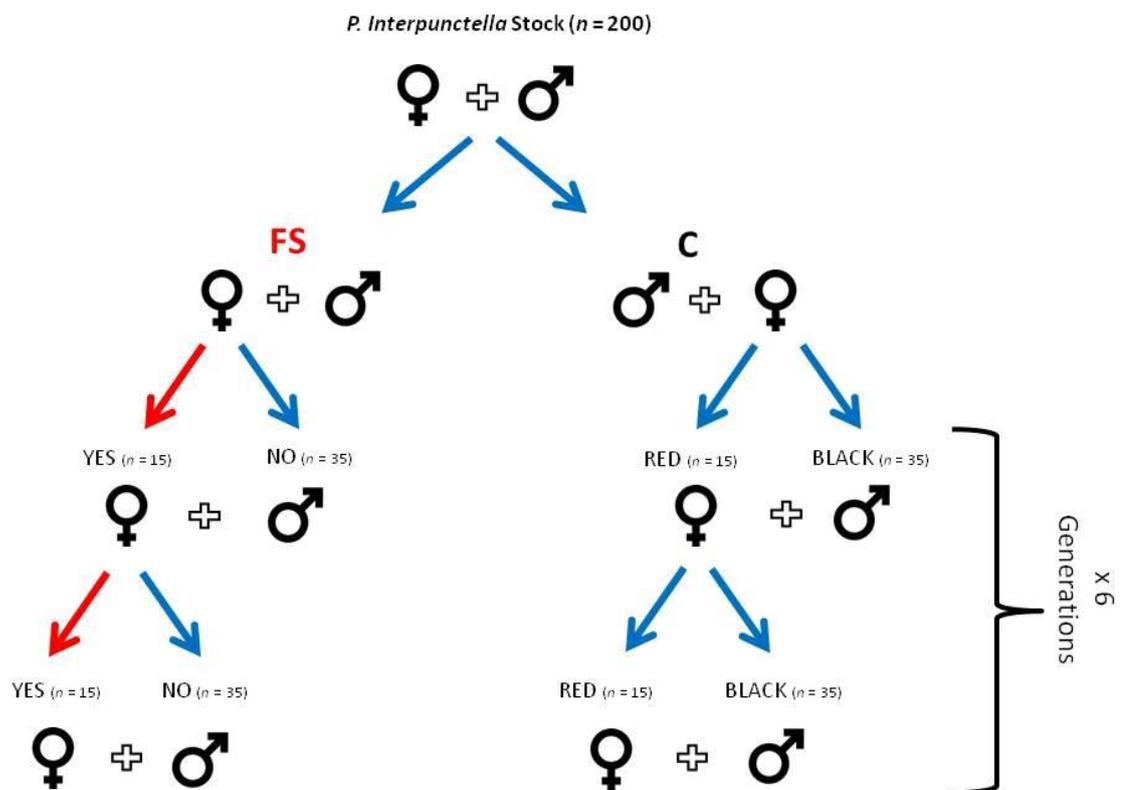


Figure 5.1: Diagram of experimental design. Using *Plodia interpunctella* stock population, larvae were randomly allocated either to *Fecundity Selected (FS)* lines, or *Control (C)* lines. Adult moths were allowed to mate on first day of eclosion (indicated by +). Eggs were counted for every generation of the *FS* lines, allowing total lifetime fecundity counts to be regressed on lifespan. The top 15 most fecund females were selected for the *Yes* treatment (red arrows indicate selection), and eggs were combined to use as focal animals for the next generation. The remaining 35 female's eggs were combined for the *No* treatment, to be used as mates in the following generation. *Control* lines were maintained by randomly selecting 15 females at each generation for the *Red* treatment (without counting eggs), which provided animals for the next generation. Eggs from the remaining 35 females were combined for the *Black* treatment, in order to provide sires. Eggs from the *C* lines were counted at generation six, when the experiment ceased. Comparisons were made within treatments, and between selection regimes, for lifetime and early fecundity after six generations of selection.

5.3.3.ii Control (C) lines

To maintain the *Control* lines, we treated focal animals in the same way as above. Focal females were randomly selected, and mated with sires from within the same selection lines. However, we did not count eggs from focal females at every generation. Instead, eggs collected from mated focal females were transferred into vials labelled according to focal female. Lifespan was recorded for each focal female from the *Control* lines. Upon death of the final female at each generation, we used a random number generator to select 15 females to pool eggs for the *Red* treatment (see Figure 5.1). The *Red* treatment is analogous to the *Yes* treatment for the *FS* lines, in that moths that came from this treatment would be focal animals in the next generation. The remaining 35 female's eggs were combined to form the *Black* treatment, which is analogous to the *No* treatment in the *FS* lines (see Figure 5.1). Eggs were pooled in a large stock pot with an excess of larval medium, and moths that developed from the *Black* treatment were used as mating partners for focal females in the next generation. To allow for comparisons to be made regarding lifetime fecundity, focal females in the control lines had their eggs counted and recorded at generation six (the end point of the experiment) in both replicates. Body size for focal females from the *Control* line was measured as above.

5.3.4 Early Fecundity

In *Fecundity Selected* (FS) lines, eggs were counted for each focal female at 3 days post-eclosion at every generation. This allowed us to test for differences in early

fecundity i.e. eggs laid in first 72 hours after eclosion and mating within treatments of the *Fecundity Selected* lines at generation six. Three days was chosen as a measure of early fecundity, as the average lifespan of females moths is around 7 days.

Females from the *Control* (C) lines had eggs counted at generation six in order to obtain early fecundity. As above, eggs were counted 3 days after eclosion and mating, which allowed us to compare between treatments of the *Control* lines, as well as across selection regimes.

5.3.5 Statistical analysis

Lifetime Fecundity

Analysis of covariance (ANCOVA) was used to compare lifetime fecundity within the *Yes* and *No* treatments from the *Fecundity Selected* (FS) lines at generation six, the end point of the experiment. ANCOVA was also used for fecundity differences within *Red* and *Black* treatments from the *Control* (C) lines at generation six. In order to take into consideration fecundity differences due to life-history traits, lifespan and body size were entered in to the model as covariates. Three outliers were removed from lifespan (1 x C *Black*, and 2 x FS *No*), and four from body size (2 x C *Black* and 2 x FS *No*) respectively, as were more than 2.5 SD away from mean. This ensured normality of life-history covariates. Replicate was nested within treatment to test for its effect in the model. The same model was used to test for differences in lifetime fecundity between treatments using *Yes* females from the FS lines and *Red* females from the C lines at generation six, when the selection experiment had ceased. Differences in two life-history traits (lifespan and body size) within treatments for *Fecundity Selected* lines (*Yes* and *No*) and *Control* lines (*Red* and *Black*), and between the selection lines were conducted using analysis of variance (ANOVA).

Early Fecundity

Fecundity counts 72 hours post-eclosion were compared between the *Yes* and *No* treatments from the *Fecundity Selected (FS)* lines at generation six. The same analysis was also conducted between the *Red* and *Black* treatments from the *Control (C)* lines also at generation six. A generalised linear model (GLM) with quasi-poisson error distribution was used as early fecundity data was non-normal and over-dispersed, and could not be transformed. Replicate was nested within treatment, and body size and lifespan were placed into the model as covariates. The same model was used to test for differences in early fecundity between treatments using *Yes* females from the *FS* lines and *Red* females from the *C* lines at generation six, when the experiment had ceased.

All statistical measures were conducted using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

5.4 Results

5.4.1 Lifetime Fecundity at Generation Six

5.4.1.i *Fecundity Selected lines:*

Lifetime fecundity was compared for *Yes* and *No* treatment females at generation six. Females from the *Yes* treatment were found to have significantly greater lifetime fecundity than females from the *No* treatment (ANCOVA, $F_{1,92} = 92.063$, $P < 0.001$; fecundity \pm 1SE *Yes*: 99.1 ± 4.287 , *No*: 48.8 ± 2.782 eggs; Figure 5.2). There was a significant effect of replicate within treatment ($F_{2,94} = 5.417$, $P = 0.006$; mean \pm 1SE Rep 1 = 67.5 ± 3.471 ; Rep 2 = 80.3 ± 3.588). Longevity showed a significant

negative correlation with lifetime fecundity ($F_{1,92} = 31.872, P < 0.001$), as females who laid more eggs had shorter lifespans. Body size was shown to significantly affect fecundity ($F_{1,92} = 5.248, P = 0.024$), as larger females had higher reproductive output.

Overall, there was no significant increase in lifetime fecundity within both treatments of the *Fecundity Selected* lines from the start of the experiment until selection ceased at generation six. Both *Yes* females (ANOVA; $F_{1,56} = 2.409, P = 0.126$; means ± 1 SE Gen 0 = 117.0 ± 5.823 , Gen 6 = 104.3 ± 5.723) and *No* females ($F_{1,137} = 3.699, P = 0.057$; means ± 1 SE Gen 0 = 59.7 ± 4.412 , Gen 6 = 47.7 ± 4.411) had similar levels of fecundity at the start of selection as they did by the end at generation six (Figure 5.2).

5.4.1.ii *Fecundity Selected* life-history traits:

Lifespan was compared between *Yes* and *No* treatments of the *Fecundity Selected* lines, and we found no significant difference in longevity (ANOVA; $F_{1,96} = 1.472, P = 0.228$). This indicates that females were selected based on fecundity, and that we did not inadvertently select on lifespan. Replicates did not differ between *Yes* and *No* treatments ($F_{2,96} = 0.656, P = 0.521$). Females from the *Yes* treatment were found to have a significantly larger body size than females from the *No* treatment ($F_{1,96} = 9.352, P = 0.003$; mean ± 1 SE *Yes*: 5.3 ± 0.049 , *No*: 5.1 ± 0.032 mm). Replicate had no significant effect on body size between the *Yes* and *No* treatments ($F_{2,96} = 1.692, P = 0.190$).

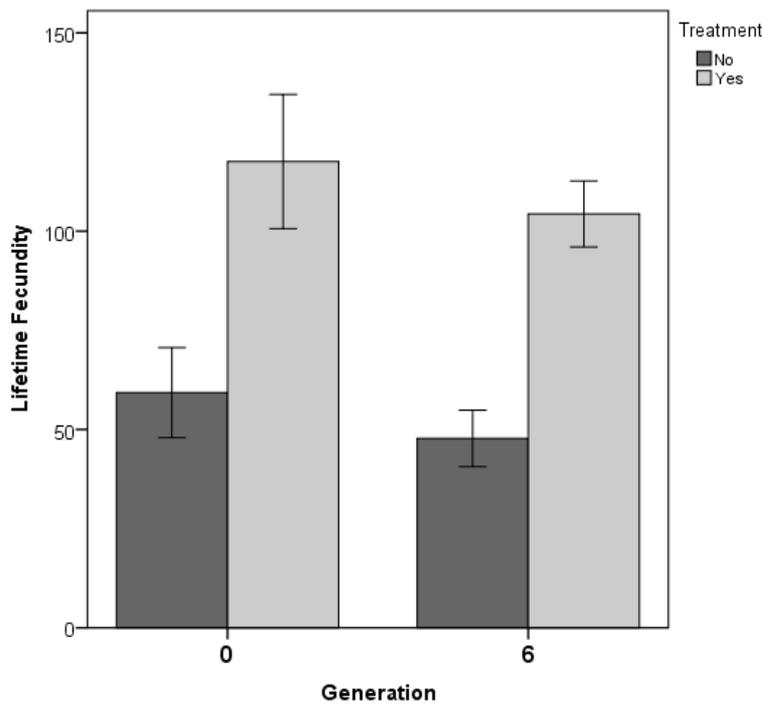


Figure 5.2: Mean lifetime fecundity for *Fecundity Selected (FS)* females at generations 0 and 6. Top 15 selected females for increased reproductive success (*Yes* treatment; light grey bars) had higher fecundity than the remaining females (*No* treatment; dark grey bars) across both replicates of *FS* lines. Error bars indicate 95% confidence intervals.

5.4.1.iii Control lines:

There was no significant difference in lifetime fecundity between *Red* and *Black* females (ANCOVA; $F_{1,95} = 0.583$, $P = 0.447$; Figure 5.3). This indicates females were selected at random with respect to fecundity. Replicate had no significant effect within treatments ($F_{2,95} = 0.271$, $P = 0.763$). As in the *Fecundity Selected* lines, longevity was found to significantly negatively correlate with fecundity ($F_{1,95} = 28.672$, $P < 0.001$). Body size did not differ between *Red* and *Black* treatments within *Control* lines ($F_{1,95} = 2.353$, $P = 0.128$).

5.4.1.iv Control line life-history traits:

There were no significant differences in lifespan between females from the *Red* and *Black* treatment within the *Control* lines (ANOVA; $F_{1,99} = 0.249$, $P = 0.620$), indicating we randomly chose females for the next generation. However, there were significant differences in lifespan between replicates ($F_{2,99} = 16.801$, $P < 0.001$; mean \pm SE Rep 1 = 9.4 ± 0.3 , Rep 2 = 11.6 ± 0.3 days). Although body size did not significantly differ between treatments in the *Control* lines ($F_{1,98} = 0.169$, $P = 0.682$), it was found that size between replicates significantly varied ($F_{1,98} = 14.216$, $P < 0.001$; mean \pm 1 SE Rep 1 = 5.4 ± 0.037 , Rep 2 = 5.1 ± 0.037 mm).

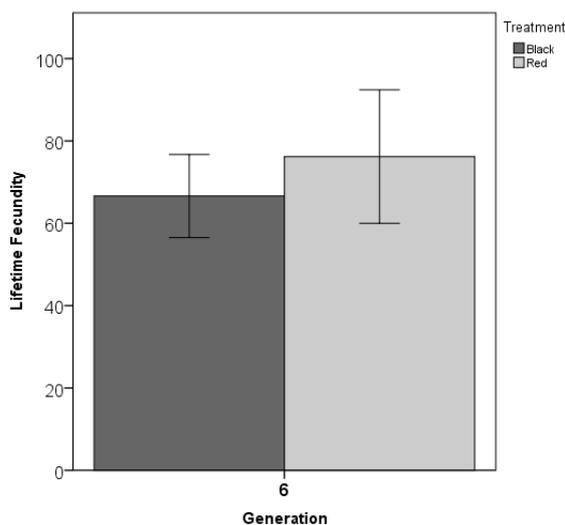


Figure 5.3: Mean lifetime fecundity for females in *Control* (C) lines at generation 6. Fifteen randomly selected females for the *Red* treatment (light grey bar) did not show any fecundity differences with the remaining females in the *Black* treatment (dark grey bar) for both replicates. Error bars indicate 95% confidence intervals.

5.4.2 Lifetime Fecundity between Selection Lines at Generation Six

We compared lifetime fecundity between the *Yes* treatment females from the *Fecundity Selected* lines with *Red* treatment females from the *Control* lines. We used the top 15 (*Yes*) females and randomly chosen 15 (*Red*) females in both replicates at generation six. By generation six, we had significantly increased lifetime fecundity in *Yes* females when compared to *Red* females (ANCOVA; $F_{1,54} = 6.523$, $P = 0.014$; means ± 1 SE *Yes*: 99.8 ± 5.176 , *Red*: 80.8 ± 5.176 eggs; Figure 5.4). There was no significant effect of replicate when nested within treatment ($F_{2,54} = 0.570$, $P = 0.43$). Again longevity was significantly negatively correlated with lifetime fecundity ($F_{1,54} = 19.669$, $P < 0.001$). Body size was also found to be significantly related to total lifetime fecundity ($F_{1,54} = 7.62$, $P = 0.008$), with increased number of eggs produced by larger females.

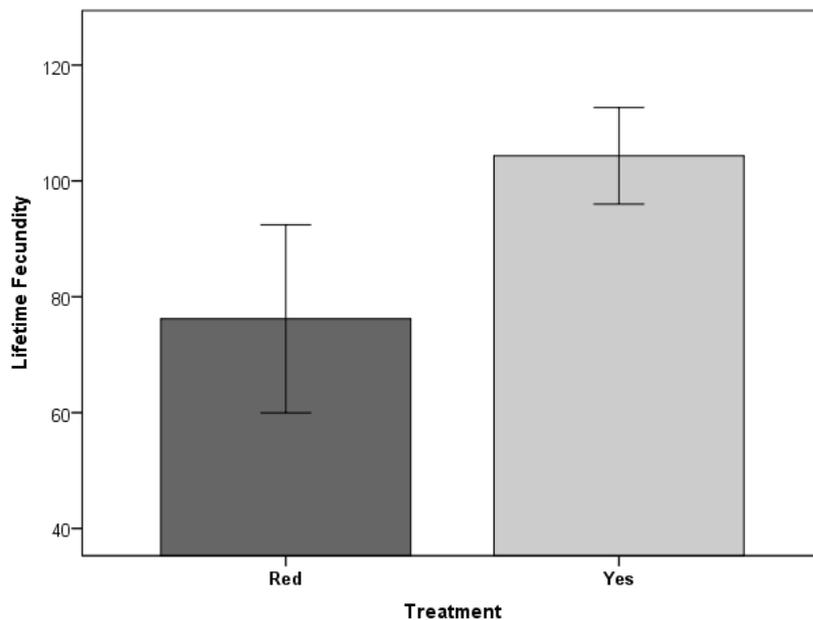


Figure 5.4: Mean lifetime fecundity for both replicates of the *Yes* treatment (top 15 most fecund) females from *Fecundity Selected* lines (light grey bar), and *Red* treatment (15 randomly selected) females from *Control* lines (dark grey bar). Lifetime fecundity was higher overall in the *Yes* treatment females. Error bars indicate 95% confidence intervals.

5.4.2.i Life-History Traits:

As longevity and body size had significant effects on lifetime fecundity, we tested for differences in these two life-history traits between *Yes* females (*Fecundity Selected* lines) and *Red* females (*Control* lines) at generation six. Overall means between selection lines showed a marginally non-significant trend for *Yes* females in *FS* lines to have shorter lifespans than *Red* females from *C* lines (ANOVA; $F_{1,56} = 3.915$, $P = 0.053$; means ± 1 SE *Yes*: 9.6 ± 0.286 , *Red*: 10.4 ± 0.286 days). There was found to be a significant effect of replicate within selection lines ($F_{2,56} = 7.569$, $P < 0.001$; means ± 1 SE *Control*: Rep 1 = 9.3 ± 0.404 , Rep 2 = 11.5 ± 0.404 ; *Fecundity Selected*: Rep 1 = 9.3 ± 0.404 , Rep 2 = 9.9 ± 0.404 days).

A comparison was made between selection lines for body size, and we found no significant differences in size between *Yes* females from *FS* lines with *Red* females from *C* lines (ANOVA; $F_{1,56} = 0.065$, $P = 0.799$). There was a significant effect of replicate within lines regards body size ($F_{2,56} = 7.569$, $P < 0.001$; means ± 1 SE *Control*: Rep 1 = 5.4 ± 0.061 , Rep 2 = 5.1 ± 0.061 ; *Fecundity Selection*: Rep 1 = 5.2 ± 0.061 , Rep 2 = 5.4 ± 0.061 mm).

5.4.3 Early Fecundity at Generation Six

5.4.3.i *Fecundity Selected* lines

A comparison of early fecundity (72 hours post- eclosion) was made within treatment for *Fecundity Selected* lines. We found that females in the *Yes* treatment laid more eggs in the first 3 days than females from the *No* treatment (GLM; $\chi^2_1 = 4.605$, $P = 0.032$; means ± 1 SE *Yes*: 13.3 ± 3.632 , *No*: 5.7 ± 1.710). Replicate had a significant effect within the treatments of the *FS* lines ($\chi^2_2 = 9.723$, $P = 0.01$; means ± 1 SE Rep 1:

5.10 ± 1.635, Rep 2: 15.28 ± 3.275). Longevity was also found to have a significant negative effect with respect to early fecundity ($\chi^2_1 = 27.073$, $P < 0.001$). In contrast, we found no significant effect of body size on early fecundity within treatments ($\chi^2_1 = 2.194$, $P = 0.139$).

5.4.3.ii Control lines

There was no significant difference in early fecundity between *Red* and *Black* females of the *Control* lines (GLM; $\chi^2_1 = 0.2$, $P = 0.888$). There were no significant differences between replicates within treatment ($\chi^2_2 = 1.245$, $P = 0.537$). Again, longevity was found to significantly negatively correlate with early fecundity within *Control* treatments ($\chi^2_1 = 30.346$, $P < 0.001$) whereas body size was found to have no significant effect ($\chi^2_1 = 0.007$, $P = 0.932$).

5.4.4 Early Fecundity between Selection Lines at Generation Six

Early fecundity was compared between selection lines, using the top 15 selected *Yes* females from both replicates of *Fecundity Selected* lines and randomly selected *Red* females from *Control* lines. Fecundity in first three days post-eclosion was significantly greater in *Red* females than *Yes* females (GLM; $\chi^2_1 = 4.156$, $P = 0.041$; means ± 1 SE *Yes*: 12.77 ± 3.534, *Red*: 21.93 ± 5.344; Figure 6.5). There was no significant effect of replicate within selection lines ($\chi^2_2 = 2.47$, $P = 0.291$). Longevity was found to have a significant negative correlation with early fecundity ($\chi^2_1 = 13.299$, $P < 0.001$) whereas body size was shown to have no significant effect on early fecundity ($\chi^2_1 = 0.011$, $P = 0.917$).

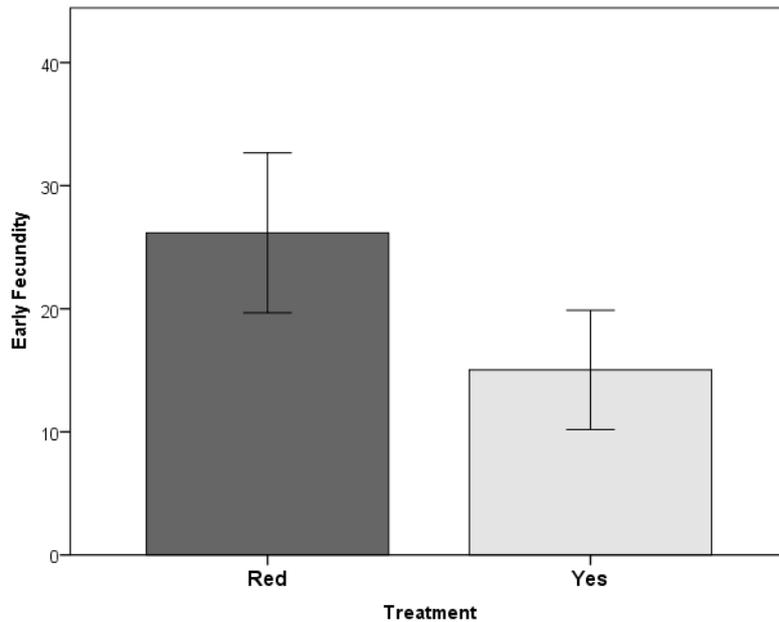


Figure 5.5: Mean early fecundity (eggs laid in first 72 hours post eclosion) in top 15 selected *Yes* females from the *Fecundity Selected* lines compared to 15 randomly selected *Red* females from the *Control* lines. Overall, *Red* treatment females laid a greater number of eggs in first 72 hours post- eclosion. Error bars indicate 95% confidence intervals.

5.5 Discussion

After multiple generations of selection on fecundity in *Plodia interpunctella*, lifetime reproductive output was successfully increased in *Fecundity Selected (FS)* lines compared to the *Control (C)* lines (Figure 5.4). This increase in fecundity was independent of changes in life-history traits known to correlate and trade-off with fecundity, such as body size and lifespan. Importantly, we found no significant differences in longevity or body size between *Yes (Fecundity Selected)* females and *Red (Control)* females. Furthermore, *Red* females in the *Control* lines were found to have

higher fecundity in the first three days after eclosion (Figure 5.5), in comparison to their *Fecundity Selected* line conspecifics.

5.5.1 Lifetime Fecundity

As predicted, lifetime fecundity was successfully increased in *Yes* treatment females from the *Fecundity Selected (FS)* lines, compared to the analogous *Red* treatment females from the *Control (C)* lines. Direct selection on fecundity after six generations resulted in *Yes (FS)* females laying around 19 more eggs over the course of their lifespan (see Figure 5.4). Previous research targeting selection directly on female fecundity in *D. melanogaster* for example has shown that, although not significant, there was a trend of upward selected females to lay more eggs compared to females from the control lines (Reeve and Fairbairn, 1999), which mirrors our findings. Our method of selecting the most fecund females after performing linear regression to control for lifespan worked, as we consistently found that *Yes* treatment females from the *FS* lines lay more eggs than *No* treatment females (see Figure 5.2) without significant differences in lifespan (discussed below). *Control* lines were maintained under random selection of *Red* and *Black* females at every generation. Although eggs were not counted at every generation, we found that at generation six there were no significant differences in overall lifetime fecundity between treatments within the *Control* lines (see Figure 5.3). We conclude that *P. interpunctella* are able to respond to female-specific selection on fecundity, with a small but significant increase in number of eggs produced over their lifetime.

5.5.2 Early Fecundity

Our findings for early fecundity (eggs laid over 3 days) again reveal differences between the selection lines. Although both selection regimes show peaks for early

fecundity, a pattern reflected in a number of Lepidoptera species such as *Spodoptera exigua*, the beet armyworm moth (Tisdale and Sappington, 2001), and *Sesamia nonagriodes*, the Mediterranean corn borer moth (Robert and Frerot, 1998) for example, we found that by the cessation of the experiment *Red* females from the *Control* lines lay around 10 more eggs early on (early fecundity) than *Fecundity Selected (Yes)* females (see Figure 5.5). Fecundity (and therefore fitness) may be condition-dependant, causing peaks in early fecundity when resources are abundant and conditions favourable. For example, early fecundity is higher in *D. melanogaster* females who are supplemented with yeast after mating, compared to female flies that received no yeast (May *et al.*, 2015). This does not apply here however, as both *Fecundity Selected* and *Control* line *P. interpunctella* females had their adult and larval conditions standardised throughout the experiment. Fertility rates are known to decline over the lifespan in insects such as *Spodoptera exigua* for example, causing fewer eggs to hatch from older females (Tisdale and Sappington, 2001). *Red* females of the *Control* lines may lay higher numbers of eggs earlier on to maximise egg hatching rates, compared to the *Yes* females of the *Fecundity Selected* lines. However, previous research has shown that fertility does not vary across lifespan in *P. interpunctella* (Cook, 1999). Higher early fecundity may be a form of bet-hedging, in case of environmental conditions becoming unfavourable in future (Berger *et al.*, 2012). It may also be speculated that *Control* line females better reflect the reproductive strategy used by *P. interpunctella* in the wild, to lay higher numbers of eggs when resources and conditions are favourable. *Control* line females are not under direct selection for increased lifetime fecundity in nature, unlike what we have done here, and hence may opt to lay the majority of their eggs early even if this means lower overall lifetime fecundity.

Despite our findings showing increased fecundity levels in the *Yes* females compared to their *Control* lines counterparts, we did not significantly increase lifetime fecundity within the *FS* line females overall at generation six compared to the start of the experiment (see Figure 5.2). It is possible that despite directed selection on increased fecundity, *P. interpunctella* females may already be at their maximum fecundity. Pest species, such as *P. interpunctella*, may be selected for maximum reproductive output, as seems to be the case in *Callosobruchus maculatus* beetles for example (Credland, 1986). *P. interpunctella* are a common pest of stored grains, with a few adults capable of rapidly producing large numbers of larvae (Williams, 1964). In order to ensure maximum numbers of larvae successfully eclosing into adult moths, it is likely that *P. interpunctella* females are favoured by having high fecundity over the course of their life. In another pest species, Reeve and Fairbairn (1999) found that fecundity in *D. melanogaster* females could not be significantly increased after twenty generations of artificial selection. In contrast, *D. melanogaster* females responded significantly to downward fecundity selection (Reeve and Fairbairn, 1999), potentially suggesting that a stronger response in *P. interpunctella* females' fecundity may have been generated had we instead selected for decreased fecundity over six generations. If fecundity is already at maximum, *P. interpunctella* females may be constrained from producing even higher number of eggs, as our research shows. In a related experiment – this time on males, artificial selection was applied upwards and downwards on male *D. melanogaster* accessory gland size, a trait that is an important determinant of male fitness. The male accessory gland produces seminal fluids and proteins transferred to the female at mating that aid sperm transfer and suppress female remating (Wigby *et al.*, 2009). Downward selection on male *D. melanogaster* accessory glands was not sufficient to significantly cause a reduction in size for multiple generations (Wigby *et*

al., 2009), suggesting there may be a minimum stable size for accessory glands to ensure successful reproduction. Selection to make accessory glands smaller may be constrained, as this would dramatically impact males' reproductive success. Conversely when conditions are optimal and resources are readily available, selection may not be able to increase a female's fecundity as egg numbers may already be at a maximum, as shown here.

5.5.3 Life-History Traits

Life-history traits frequently correlate with fecundity in insects (Nylin and Gotthard, 1998). We measured two life-history traits known to affect fecundity, body size and lifespan. A consistent negative correlation between lifespan and lifetime fecundity was observed in both *Fecundity Selected* and *Control* lines, in line with previous research in *P. interpunctella* (Lewis *et al.*, 2011), as well as other insects (Honěk, 1993). Resources are limited in *P. interpunctella* that do not feed as adults, and like most other insects these resources are allocated towards reproduction at the expense of somatic maintenance (Partridge *et al.*, 2005). A trade-off was evident despite controlling for the effect of lifespan on fecundity at every generation, in order to select females with highest fecundity irrespective of lifespan in the *FS* lines. Importantly, we did not inadvertently select for increased lifespan, as we found no significant differences in lifespan between *Yes* and *No* females of the *FS* lines at the end of the experiment. A comparison of longevity between our selection lines revealed a marginally non-significant trend for females in the *Yes* treatment (*FS* lines) to live shorter lifespans than females in the *Red* treatment (*Control* lines). Despite significantly increasing fecundity in the *Yes* treatment, we found no corresponding

changes to lifespan, indicating direct selection on fecundity occurred without affecting the trade-off with longevity, possibly indicating that the two traits may be uncoupled.

Body size was measured within and between selection lines in order to compare any correlated changes after increased selection on fecundity. It was found that *Yes* females from the *FS* lines had a larger body size on average than females in the *No* treatment. This corroborates previous research in many insects, showing that fecundity scales with female size (Honěk, 1993). A similar experiment selecting upwards on fecundity in *D. melanogaster* also revealed positively correlated changes in body size, despite no significant increase in fecundity (Reeve and Fairbairn, 1999). An increase in body size allows females to either accrue more resources for egg production (García-Barros, 2000), or accommodate increasing numbers of eggs (Preziosi *et al.*, 1996, and Winkler *et al.*, 2012). In contrast, *Yes* females were not significantly different in size compared to their *Red* female counterparts in the *Control* lines at generation six. This suggests that we increased lifetime fecundity in *FS* lines without a correlated response in body size.

Overall, there was a modest increase in lifetime fecundity in *Yes* females from the *Fecundity Selected* lines, which occurred without a significant increase in body size, or decrease in lifespan compared to the *Control* line females. Although body size and lifespan are correlated and traded-off with fecundity, they may not be under direct fecundity selection. These two life-history traits may each be partly decoupled from their effects on fecundity. Similarly, targeting selection on lifespan in inbred lines of *D. melanogaster* caused no differences in early fecundity, suggesting that lifespan and early fecundity are uncoupled (Khazaeli and Curtsinger, 2013; Wit *et al.*, 2013). Pupal mass is also found to be uncoupled from lifespan in the *Bicyclus anynana* butterfly (Pijpe *et al.*, 2006). Our results therefore suggest that direct selection on fecundity

does not necessarily result in corresponding changes in longevity and body size, two of the three life-history traits known to be genetically correlated with lifetime fecundity in *P. interpunctella* (Lewis *et al.*, 2011). An alternative explanation for the lack of significant body size and longevity differences between *Yes* and *Red* females may be due to the increased reproductive effort seen in both selection lines. Lifetime fecundity is increased in *Fecundity Selected* lines, and early fecundity in the *Control* lines, suggesting that egg laying patterns have shifted between the two selection regimes. Both these increases in fecundity may result in trade-offs with lifespan, and also correlate with body size, causing no observable differences these two life-history traits between selected and unselected females.

5.5.4 Conclusion

In conclusion, we have shown that lifetime fecundity in *Plodia interpunctella* can be modestly increased in response to direct selection. Our results add to the limited research regarding artificial selection for increased fecundity, particularly for insects. We also find that unselected females may adopt a reproductive strategy favouring higher early fecundity possibly in response to better conditions and favourable resources in the laboratory. A shift in egg laying patterns may have occurred as a consequence of direct selection, as early fecundity and lifetime fecundity differ between selected and unselected individuals. Two life-history traits, body size and longevity, did not significantly differ between selected and unselected females of the *Fecundity Selected* and *Control* lines respectively. More research is required to examine if genetic uncoupling of these traits from fecundity has occurred, or if reproductive output is already selected to be maximised in this pest moth species,

causing no differences in life-history traits to be observed despite modest increases in lifetime fecundity.

6. Sex-specific effects of dietary protein and carbohydrate does not resolve intralocus sexual conflict in *Plodia interpunctella*.

6.1 Abstract

Intralocus conflict occurs when alleles expressed in both sexes are subject to sexually antagonistic selection for sex-specific traits and fitness optima. Life-history traits are likely to be subject to conflict as they are shared between the sexes, have a strong relationship to fitness, and often have differing optima in the sexes. Diet is likely to be a source of conflict between the sexes, as differing macronutrients may be optimal for their respective life-history traits and reproductive success. Here we used the geometric framework to compare the effects of macronutrients in the Indian meal moth *Plodia interpunctella*, a species known to be under strong sexually antagonistic selection for shared life-history traits and fitness. By rearing first instar larvae on 24 artificial diets differing in protein and carbohydrate ratios and content, we compared survival, life-history traits and fitness within and between the sexes. We found that there were sex-specific effects of macronutrients on shared life-history traits (development time, longevity and body size). Sex-specific effects of diets on reproductive success were in opposing regions of the nutrient landscape for *P. interpunctella*, indicating intralocus conflict was not resolved despite the nutrient space available.

6.2 Introduction

Sexual conflict may arise when males and females invest differently towards reproductive strategies (Parker, 1979), as a consequence of anisogamy (Schärer *et al.*, 2012). Sexual conflict can be divided into two main forms, interlocus and intralocus. Interlocus sexual conflict occurs due to interactions of alleles at different loci between the sexes over reproduction. Interlocus sexual conflict can lead to males having higher fitness optima at the expense of female fitness, and *vice versa* (Chapman *et al.*, 2003), potentially leading to co-evolutionary arms races between the sexes (Parker, 1979). Intralocus (IASC hereafter) conflict arises due to the same alleles being expressed in both sexes but having opposing fitness effects. Many traits share a common genetic basis in males and females, but are subject to opposing patterns of selection, termed sexually antagonistic alleles (Arnqvist and Rowe, 2002; Price and Hosken, 2007). If shared phenotypic traits are selected differently in the two sexes, we may see one sex being driven away from its phenotypic optimum while the other sex shows increased fitness, generating a genomic tug-of-war (Van Doorn, 2009). IASC is thought to be at its strongest when optimal trait values are opposing in the sexes, and the intersexual genetic correlations (r_{mf}) for these traits is strong between males and females (Bonduriansky and Chenoweth, 2009). Previous research has demonstrated the action of IASC in both wild and laboratory-adapted populations over fitness (e.g. fruit flies, Chippindale *et al.*, 2001; red deer, Foerster *et al.*, 2007; side-blotched lizard, Svensson *et al.*, 2009; Indian meal moth, Lewis *et al.*, 2011).

Life-history traits are likely to be subject to high levels of sexual conflict, given their strong relationship to fitness, the fact they are shared between the sexes, and often have differing optima in the sexes (Wedell *et al.*, 2006). A recent study on the

Indian meal moth, *Plodia interpunctella*, demonstrated multivariate selection acting on three shared life-history traits (development time, longevity and body size) that are strongly associated with fitness in both sexes (Lewis *et al.*, 2011). Opposing selection act on these shared life-history traits, as evident through trait combinations that were favourable to female fitness were detrimental to male fitness, and *vice versa*. In male *P. interpunctella*, high fitness was optimised through rapid development time and longer lifespan. In contrast, female fitness was maximised by longer development time, larger body size and shorter lifespan (Lewis *et al.*, 2011). This sexually antagonistic selection is the signature of IASC (Bonduriansky and Chenoweth, 2009; Pennell and Morrow, 2013).

Life-history traits may be optimised depending on the condition of the individual. According to life-history theory, resources are limited within individuals, and allocation to one trait cause reduced investment in other traits (Zera and Harshman, 2001). Food availability or shortages whilst developing can impact on life-history traits in insects, such as body size and development times as in the American rubyspot damselfly, *Hetaerina Americana* for example (Jiménez-Cortés *et al.*, 2012). Condition of an individual can be experimentally manipulated through altering diet quality (e.g. Hunt *et al.*, 2004; Hall *et al.*, 2008), or through caloric restriction (e.g. Adler *et al.*, 2013). Effects of diet may be sex-specific, with dietary restriction widely recognised as a method that increases lifespan, yet reduces female fecundity (Partidge *et al.*, 2005; Nakagawa *et al.*, 2012; Adler *et al.*, 2013). Higher quality diets can lead to increased sperm numbers in males, such as in *P. interpunctella* (Gage and Cook, 1994), and red flour beetles, *Tribolium castaneum*, where higher paternity success was obtained as a result of modified diet changes (Fedina and Lewis, 2006).

Traditionally diets have been manipulated through addition or subtraction of a key ingredient. Nitrogen limitation in larvae of the small white butterfly, *Pieris rapae* for example, is shown to adversely affect resources allocated to egg production (Tigreros *et al.*, 2013). Instead, nutritional geometry is now being employed as a tool by ecologists. Nutritional geometry, or the Geometric Framework (Simpson and Raubenheimer, 1993), allows for specific nutrients in the diet to be monitored in their effects on trait expression and fitness in a species. Animals can be restricted to artificial, holidic (chemically defined) diets that vary in both nutrient concentration and ratio (Simpson and Raubenheimer, 2012). A multidimensional landscape is produced, allowing visualisation of both linear and nonlinear effects of monitored nutrients, notably carbohydrates and proteins, on specific traits of interest in individuals (Simpson and Raubenheimer, 2009). A number of studies have tested for effects of dietary protein and carbohydrates on range of traits, including male sex pheromone expression in the cockroach *Nauphoeta cinerea* (South *et al.*, 2011), sperm number and viability in the cockroach *N. cinerea* (Bunning *et al.*, 2015), and lifespan and reproduction in *Drosophila melanogaster* (Lee *et al.*, 2008). Only a handful of studies have set out to examine the specific effect of nutrient consumption between the sexes on lifespan and fitness, two traits that are known to be differentially selected in males and females (Maklakov *et al.*, 2008; Jensen *et al.*, 2015). For example, when given a choice over two combinations of diets, the field cricket *Telogyllus commodus* favoured diets that were more beneficial for sex-specific reproduction over lifespan extension. Furthermore, due to genetic correlations between males and females may prevent each other from obtaining the correct nutrients to increase their relative fitness optima, indicating sexual conflict over diet choice (Maklakov *et al.*, 2008).

The Indian meal moth, *Plodia interpunctella*, is a tractable study species as they are a global pest of stored grains. This allows the conditions that they are found in the wild to be easily replicated in the laboratories. *P. interpunctella* are known to be under intralocus sexual conflict for shared life-history traits (development time, longevity and body size), with opposing trait combinations being favourable in one sex at the expense of the other (Lewis *et al.*, 2011). These shared traits are strongly correlated with fitness, so as male fitness was maximised, the same trait combination was simultaneously detrimental to female fitness, and *vice versa*. In male *P. interpunctella*, high fitness was optimised by rapid development time and longer lifespan. Given that male fitness was dependent on achieving successful copulations, a rapid development time and longer lifespan would allow males to increase their chances of finding a mating partner, thus increasing the numbers of mating events (Lewis, 2005). Mating success in male *P. interpunctella* moths has been shown to be unaffected by body size (Cook *et al.*, 1997). In contrast, female fitness was maximised by a long development time, shorter lifespan and a large body size. Large size in female insects has been shown to correlate with increased fecundity that is frequently traded off against lifespan (Honěk, 1993). Increased fecundity may have a knock-on effect on development time, as more fecund females may need a longer period to acquire resources needed for egg production during the larval development stage (Nylin and Gotthard, 1998). Unlike previous species to be studied using nutritional geometry, *P. interpunctella* are capital breeders, meaning all energy required for reproduction and allocation to fitness related traits must be acquired when feeding as larvae (Jönsson, 1997).

In this study we used nutritional geometry to examine the effects of protein and carbohydrate consumption on shared life-history traits subject to intralocus sexual

conflict, and fitness in *P. interpunctella*. Larvae were reared on diets from first instar; in contrast to other studies that only used fifth instar lepidopteran larvae to examine the effects of protein and carbohydrates in capital breeders (e.g. Telang *et al.*, 2001; 2003; Lee, 2010). To our knowledge, only one previous study has attempted to determine protein and carbohydrate effects from first instar through to egg production as adults - the moth *Heliothis virescens* (Roeder and Behmer, 2014). However, only fecundity and egg viability were determined for mated pairs, and no quantification of male mating success, a key measure of reproductive performance was performed (Roeder and Behmer, 2014). Our study is the first of its kind to examine larval survival, measures of shared life-history traits in the sexes, as well as determining female fecundity, and male mating success after raising larvae on 24 diets available in the geometric framework. In order to test for sex-specific effects of dietary protein and carbohydrate, nutritional landscapes were compared between males and females. This allowed us to determine if conflict over shared life-history traits may be resolved across the expansive nutrient space available.

6.3 Methods

6.3.1 Stock Populations

See Chapter 5; section 5.3.2.

6.3.2 Experimental animals

Larvae were randomly collected from the stock population to be used as experimental animals. In order to generate the large numbers of larvae required as experimental animals and as standard mating partners for the fitness assays, an

increased breeding regime was undertaken for two generations. Roughly 800 males and 800 females were collected at 4th instar and housed in single sex pots. Larvae can be sexed easily around 4th- 5th instar, due to the pigmented testes in males being visible through the body cavity wall (Ingleby *et al.*, 2010). Upon eclosion, adult males and females were combined together for copulation in an egg collector. An egg collector is a modified 1L stock pot that has black mesh at the bottom, secured in place with an elastic band. The inverted stock pot is then taped into a funnel, which in turn is secured into a conical flask. This allows males and females to interact together, and any eggs laid in this period to be deposited at the bottom of the conical flask, ready for transfer to new larval medium. Adults were allowed to mate and interact for 3- 4 days. After each 24 hour period, the conical flask containing the resulting eggs was removed, and replaced with a new flask. The eggs were evenly distributed across a number of small petri dishes (5 cm diameter). Two petri dishes containing eggs were placed inside another sealed container (11 x 11 x 3 cm) in order to minimise escape when larvae hatched. Each container was labelled according to date of egg collection (in order to establish development time). Containers were placed back into an incubator (on same L:D and temperature cycle as above), and monitored every day for hatching larvae, roughly 4- 5 days after eggs had been collected. Newly hatched first instar larvae were then transferred to the diets outlined below.

6.3.3 Feeding Protocol

Twenty-four artificial diets that varied in ratio of Protein:Carbohydrate (P:C) were made for use in this experiment. Diets varied across 6 nutritional rails, based on a protocol outlined by Simpson and Abisgold (1985). Each rail varied in ratio of P:C with 4 different concentrations within each rail of total amount of P and C (i.e. nutrient

concentration). The composition of these diets can be found in appendix Table 6.1A. After a period of preliminary testing of diets with *P. interpunctella* larvae, we found that powdered diets mixed with glycerol resulted in higher survival/ eclosion rate of adults. Glycerol is a simple sugar alcohol, which has a sweet taste, and low toxicity, and is used as one of the main ingredients in the larval medium mixed with bran midlings. Powdered diets were mixed with glycerol at a ratio of 5:1 (i.e. 5g of diet to 1g of glycerol). All diets were mixed and placed in -20°C freezer until start of feeding commenced. Around 2- 3g of each diet was then placed into 30mL Sterilin vials ready for 1st instar larvae transfer. Larvae were fed ad-libitum throughout the experiment.

Upon hatching into 1st instar, larvae were randomly allocated to one of the 24 diets. A total of 20 replicates were used for each diet, totalling $n = 480$ vials. Upon hatching from eggs (already contained in sealed and dated boxes), individual larva were selected at random and transferred using a fine-tipped paintbrush into randomly selected vials containing the pre-mixed diets. This process was repeated until there were 20 larvae in each vial. As *P. interpunctella* larvae live in and amongst their food, larvae were left to develop, and monitored until the sexes could be identified, from 4th instar onwards. Larvae were then separated according to sex into single sex vials containing pre-mixed granular diet, to ensure virginity upon eclosion. Vials were then monitored daily until eclosion of adult moths.

6.3.4 Shared Life-History Traits

See Chapter 2; section 2.3.3.

Where possible, we aimed to obtain measures from 5 animals per sex, per replicate for the three shared life-history traits (development time, longevity and body size) across all diets.

6.4.5 Fitness Assays

See Chapter 2; section 2.3.4.

We aimed to measure reproductive success from 1 animal of each sex, per replicate, across the 24 artificial diets. Mating partners for both female and male fitness measures were collected from stock pots.

6.3.6 Statistical Analysis

The linear, nonlinear (quadratic), and correlational effects of P and C intake on shared life-history traits (development time, longevity and body size) and fitness were quantified using a multivariate response-surface approach (Lande and Arnold, 1983). Non-parametric thin-plate splines were used to visualise the nutritional landscape for each of the three shared life-history traits (development time, longevity and body size), and fitness. Prior to analysis, we standardized each response variable and nutrient intake to a mean of zero and standard deviation of one using a Z-transformation to ensure that our regression gradients were presented in the same scale. These were constructed using the *Tps* function in the 'FIELDS' package using R version 2.1; R Development Core Team (2008). Negative quadratic terms of P x P and C x C indicate peaks in the nutritional landscape, whilst positive quadratic terms indicate troughs. The larger the nonlinear term, the steeper the peak/ trough observed in the landscape (i.e. 0.9 is steeper than 0.2).

A sequential model building approach was used to assess whether the linear and nonlinear effects of protein and carbohydrate differed between the sexes for the three shared life-history traits and fitness (South *et al.*, 2011). Significant differences in these model comparisons were detected using univariate tests to demonstrate the linear, quadratic and correlational effects of specific nutrients (P or C) on the shared

life-history traits and fitness respectively. In order to compare if optimal life-histories and fitness peaks in the same areas of the nutritional landscape, the angle (ϑ) between the linear vectors of the response variables being compared was also calculated (Bunning *et al.*, 2015). After calculating angles between shared life-history trait and fitness landscapes, angle estimates were produced of mean, median, minimum and maximum angles. The 1st and 3rd quantiles are functionally equivalent to 95% CI's. We use the median and 95% CI's when reporting results. If the angle $\vartheta = 0^\circ$, then the vectors are aligned, and optimal peaks occupy the same area of the nutrient landscape. However if $\vartheta = 180^\circ$, then optimal peaks are in complete divergence from each other.

6.4 Results

6.4.1 Survival

The content of both Protein (P) and Carbohydrate (C) in the diet had significant linear, quadratic and correlational effects on the proportion of larvae surviving until eclosion as adult moths (Figure 6.1). Survival of larvae was linearly affected through both P content (0.34 ± 0.03 , $t_{479} = 12.34$, $P = 0.0001$), as well as C content, although more of a gradient was generated with consumption of C (0.78 ± 0.03 , $t_{479} = 27.87$, $P = 0.0001$). Significant quadratic effects revealed a small peak for survival with P content (-0.16 ± 0.02 , $t_{479} = 7.32$, $P = 0.0001$), and a significant quadratic effect of C content (0.15 ± 0.02 , $t_{479} = 6.58$, $P = 0.0001$). Inspection of the nutritional landscape indicates that optimal P and C content for larval survival occurs approximately at a P:C ratio of 1:2 (Figure 6.1). Overall, there was a significant correlational effect of P and C content

(0.75 ± 0.04 , $t_{479} = 18.28$, $P = 0.0001$), indicating that both nutrients were important, but a higher content of C led to higher proportion of surviving larvae.

In summary, both nutrients are required for larval survival, but a higher proportion survived on diets with a higher concentration of C, approximately a 1:2 ratio of P:C.

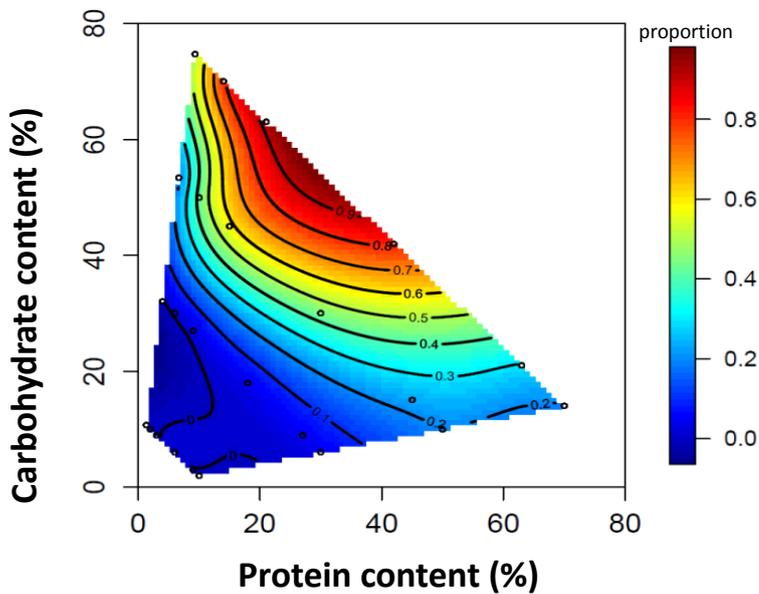


Figure 6.1: The nutritional landscape for proportion of larvae surviving until eclosion as adult moths. Red regions of the landscape indicate higher proportions of larval survival, whilst the blue regions represent lower proportions.

6.4.2 Shared life-history traits within the sexes

6.4.2.i Males

The content of both Protein (P) and Carbohydrate (C) in the diet had clear linear and nonlinear effects on the development times of surviving males (Table 6.1 and Figure 6.2a). Shorter development times were achieved with positive linear

content of both nutrients for males (Table 6.1). Significant quadratic terms indicate a peak in development time (Table 6.1). Inspection of the nutritional landscape showed that the peak for development time occurred at an approximate ratio 1:1 of P:C (Figure 6.2a). There was also a significant positive correlational gradient (Table 6.1), corroborating that an increase of both nutrients caused shorter development times in males being favoured.

P and C content of diets had linear and nonlinear effects on male body size (Table 6.1 and Figure 6.2c). Body size of males was increased with content of both nutrients, although more of a response was generated by C content than P in the diet (Table 6.1). There was a quadratic effect of C content, causing a peak for body size (indicated by a negative coefficient; Table 6.1). Inspection of the nutritional landscape shows a peak in body size occurred at an approximate ratio of 1:2 P:C (Figure 6.2c), indicating that an increase of C content in the diet would produce a greater response in body size of males. There was no significant quadratic effect of P on body size in males, as well as no significant correlational gradient (Table 6.1).

The C content of diets caused significant linear and nonlinear effects on lifespan of males (Table 6.1 and Figure 6.2e). Higher content of C led to longer lifespans for males, in contrast to P content which had a marginally non-significant linear effect (Table 6.1). Quadratic effects of C content were significant, indicating a peak in the nutritional landscape for male lifespan to be at a ratio of approximately 1:2 P:C (Figure 6.2e). C content is more important for male lifespan extension than P as a dietary nutrient. There was no significant quadratic effect of P on lifespan in males, as well as no significant correlational gradient (Table 6.1).

In summary, equal ratios of P and C led to shorter development times in males (Figure 6.2a). Longer lifespan and larger body size in males were obtained on diets with a higher content of C, as corroborated with a 1:2 ratio of P:C (Figure 6.2c and e).

6.4.2.ii Females

Content of both Protein (P) and Carbohydrate (C) had clear linear and nonlinear effects on female development time (Table 6.1 and Figure 6.2b). Development times were shorter with linear content of both nutrients in diets (Table 6.1). Significant quadratic terms indicate a peak in development time (Table 6.1), with inspection of the nutritional landscape showing that this peak occurred at an approximate 1:1 ratio of P:C (Figure 6.2b). There was also a significant positive correlational gradient (Table 6.1), which corroborates that a higher content and equal ratio of both P and C led to shorter development times in females.

Content of both P and C had linear effects on female body size, although more of a response was generated through a higher content of C in the diet than P (Table 6.1 and Figure 6.2d). There was a significant quadratic effect of C content, indicating a peak for body size (Table 6.1). Inspection of the nutritional landscape indicates that larger female body sizes were obtained on diets containing an approximate 1:2 ratio of P:C (Figure 6.2d). There were no significant quadratic effects of P on body size, as well as no significant correlational gradient (Table 6.1).

The content of C in the diet had a significant linear effect on longevity for females, in contrast to P content (Table 6.1 and Figure 6.2f). Diets with a higher content of C in led to longer lifespans in females (Table 6.1). There were significant quadratic effects of both P and C, indicating a peak in lifespan (Table 6.1), with inspection of the nutritional landscape showing the peak occurred at a 1:5 ratio of P:C

(Figure 6.2f). There was a significant negative correlational effect of P and C (Table 6.1), due to opposing signs in linearity of the effect of these nutrients. P had no effect on female lifespan, whilst a much higher ratio of C content generated a positive response for longer lifespans in females.

In summary, equal ratios of P and C led to shorter development times in females (Figure 6.2b). Twice as much C content in the diet compared to P content led to larger body sizes in females (Figure 6.2d). Longer lifespan in females was favoured by a larger increase in C content, as corroborated by a 1:5 ratio of P:C (Figure 6.2f).

Table 6.1. The linear and nonlinear effects of protein (P) and carbohydrate (C) content in diets on shared life-history traits (development time, body size and longevity) in (A) male and (B) female *Plodia interpunctella*. Significant *P* values are highlighted in **bold**.

Response variables	Linear effects		Nonlinear effects		
	P	C	P x P	C x C	P x C
(A) Males					
Development time*					
Coefficient ± SE	0.90 ± 0.07	0.94 ± 0.07	-0.28 ± 0.08	0.33 ± 0.08	0.60 ± 0.15
<i>t</i> ₂₁₈	12.53	13.52	3.37	4.15	4.01
<i>P</i>	0.0001	0.0001	0.001	0.0001	0.0001
Body size					
Coefficient ± SE	0.47 ± 0.08	0.75 ± 0.08	0.11 ± 0.11	-0.33 ± 0.11	0.17 ± 0.21
<i>t</i> ₂₁₈	5.71	9.36	0.95	3.06	0.83
<i>P</i>	0.0001	0.0001	0.34	0.002	0.41
Longevity					
Coefficient ± SE	0.18 ± 0.10	0.34 ± 0.09	0.08 ± 0.14	-0.42 ± 0.13	-0.14 ± 0.25
<i>t</i> ₂₁₈	1.90	3.70	0.60	3.27	0.58
<i>P</i>	0.06	0.0001	0.55	0.001	0.56
(B) Females					
Development time*					
Coefficient ± SE	0.97 ± 0.06	1.04 ± 0.06	-0.41 ± 0.07	0.19 ± 0.07	0.28 ± 0.12
<i>t</i> ₂₂₅	15.45	17.34	6.09	2.85	2.26
<i>P</i>	0.0001	0.0001	0.0001	0.005	0.025
Body size					
Coefficient ± SE	0.20 ± 0.08	0.62 ± 0.08	-0.01 ± 0.10	-0.40 ± 0.10	0.16 ± 0.19
<i>t</i> ₂₂₅	2.40	7.92	0.10	3.90	0.87
<i>P</i>	0.017	0.0001	0.92	0.0001	0.39
Longevity					
Coefficient ± SE	-0.02 ± 0.09	0.26 ± 0.09	-0.29 ± 0.12	-0.69 ± 0.12	-0.99 ± 0.23
<i>t</i> ₂₂₅	0.23	2.89	2.33	5.51	4.37
<i>P</i>	0.82	0.004	0.021	0.0001	0.0001

* The sign of standardized development time is inverted to put it on the same scale as other traits (i.e. a shorter development time is good) for statistical comparison.

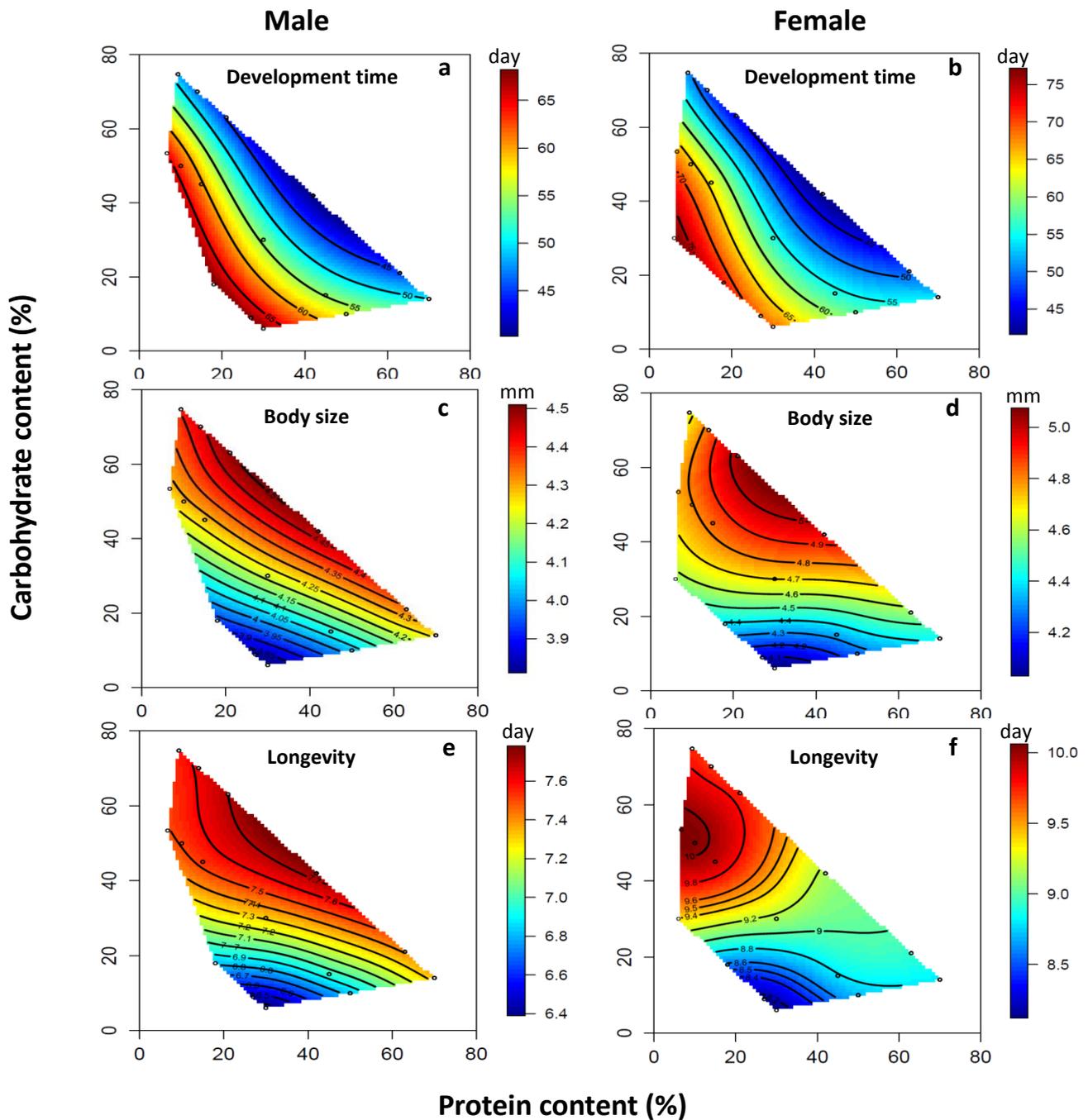


Figure 6.2: Nutritional landscapes illustrating the effects of dietary protein and carbohydrate content for males (a, c, and e) and females (b, d, and f), for the three shared life-history traits (development time, body size and longevity). In each landscape, red regions represent higher values for traits, whilst blue regions represent lower values for the traits. Note that as shorter development times are favoured, the optimal region is indicated in the blue region of the landscape.

6.4.3 Life-history Trade-offs

6.4.3.i Males

Using a sequential model based approach, nutritional landscapes of life-history traits among males were compared for trade-offs due to P and C content. A comparison between development time and body size in males revealed significant differences in P and C content for linear and nonlinear effects (Table 6.2). The difference in linear gradients was due to the fact that development time was more responsive to P content than body size ($F_{1,432} = 15.02$, $P = 0.0001$; Table 6.1), whereas C content was marginally non-significant for both traits ($F_{1,432} = 3.09$, $P = 0.08$; Table 6.1). There was a marginally non-significant correlational effect of P and C (Table 6.2), indicating a similar gradient for P and C in development time and body size in males. The peaks in development time and body size in males occurred in approximately the same region, as evident by a moderately large angle ($\vartheta = 28.5^\circ$; Table 6.2) between them (Figure 6.2a and c).

A comparison of development time and longevity within males (Table 6.2) again revealed that significant differences in linear effects was due to development time being more responsive to P ($F_{1,432} = 35.95$, $P = 0.0001$), as well as C content ($F_{1,432} = 26.54$, $P = 0.0001$) compared to lifespan (Table 6.1). Significant quadratic effects were due to shorter development times reached through a peak in P content, whilst a longer lifespan peaked with higher content of C in the diet (Table 6.1). Correlational effects of P and C on development time and longevity were due to differing gradients, as high contents of P caused a reduction in lifespan (Table 6.1). Observed peaks for these two life-history traits occur in approximately the same region of the landscape, with a moderately large angle ($\vartheta = 35.2^\circ$; Table 6.2) found between them (Figure 6.2a and e).

Finally, only linear effects of P and C were found to differ between body size and longevity in males (Table 6.2). Both P ($F_{1,432} = 5.31, P = 0.02$) and C ($F_{1,432} = 11.17, P = 0.0009$) content significantly affected both traits, yet a stronger gradient was observed for body size in response to both nutrients (Table 6.1). Both quadratic and correlational effects of P and C content were non-significant between lifespan and body size in males (Table 6.2). Comparison of the nutritional landscapes revealed that peaks for longevity and body size occurred in the same region (Figure 6.2c and e), as evident by the small angle ($\vartheta = 14.3^\circ$; Table 6.2) between the two linear vectors.

6.4.3.ii Females

Linear and quadratic effects of P and C on female development time and body size differed significantly (Table 6.2). Both P ($F_{1,446} = 55.57, P = 0.0001$) and C content ($F_{1,446} = 17.70, P = 0.0001$) caused stronger gradients to be observed for female development time compared to body size (Table 6.1). Quadratic terms revealed differing nutrients responsible for the peaks, with a significant peak in P content for development time ($F_{1,442} = 34.90, P = 0.0001$; Table 6.1), in contrast to a peak for body size due to C content ($F_{1,442} = 54.48, P = 0.0001$; Table 6.1). Inspection of the nutritional landscapes for development time and body size in females (Figure 6.2b and d) indicates that peaks of the linear vectors are diverging apart, due to a large angle ($\vartheta = 42.6^\circ$; Table 6.2) between them.

Development time and longevity were compared within females (Table 6.2), and found that significant differences in linear effects were due to stronger response in both P ($F_{1,446} = 77.75, P = 0.0001$) and C content ($F_{1,446} = 53.79, P = 0.0001$) on development time for females (Table 6.1). Quadratic terms indicates differing peaks for the two nutrients, with shorter development times peaking on diets with higher P

content ($F_{1,442} = 53.79$, $P = 0.0001$; Table 6.1), whilst longer lifespans were achieved with higher C consumption ($F_{1,442} = 13.40$, $P = 0.0003$; Table 6.1). Significant correlational effects were observed between the two nutrients (Table 6.2), due to a stronger gradient for lifespan compared to development time in females (Table 6.1). Peaks for development time and longevity occurred in differing regions of the landscape (Figure 6.2b and f), corroborated by a larger angle between them ($\vartheta = 56.3^\circ$; Table 6.2) indicating a trade-off between these life-history traits over macronutrients.

Finally, a comparison between linear and nonlinear effects of P and C showed significant differences between body size and longevity in females (Table 6.2). A stronger gradient caused larger body sizes in response to C content ($F_{1,446} = 9.46$, $P = 0.0022$), whilst there was marginally non-significant difference in P ($F_{1,446} = 3.10$, $P = 0.08$) content between the two life-history traits (Table 6.1). Significant quadratic effects were due to stronger peaks obtained for both P ($F_{1,442} = 4.42$, $P = 0.036$) and C ($F_{1,442} = 5.08$, $P = 0.025$) content for longevity over body size (Table 6.1). The differences in correlational gradients were due to a stronger response between the two macronutrients for longevity compared to body size (Table 6.1). However, a comparison of the nutritional landscapes (Figure 6.2b and f) revealed that peaks for body size and development time occurred in the same region, as illustrated by the small angle ($\vartheta = 15.9$; Table 6.2) between the two linear vectors.

Table 6.2. Sequential model based approach comparing the linear, quadratic and correlational effects of protein (P) and carbohydrate (C) content in diet on different life-history traits within the sexes of *Plodia interpunctella*. Significant P values are highlighted in **bold**.

	<i>SS_R</i>	<i>SS_C</i>	<i>DF₁</i>	<i>DF₂</i>	<i>F</i>	<i>P</i>	<i>θ</i> (95%CI)
A. Males							
Development time vs. Body size							
Linear	278.64	267.43	2	432	9.05	0.0001^A	28.5 (21.7, 35.8)
Quadratic	233.07	200.63	2	428	34.60	0.0001^B	
Correlational	196.43	195.13	1	426	2.84	0.09	
Development time vs. Longevity							
Linear	345.03	317.88	2	432	18.45	0.0001^C	35.2 (22.8, 49.9)
Quadratic	295.13	255.87	2	428	32.84	0.0001^D	
Correlational	254.39	250.49	1	426	6.64	0.01	
Body size vs. Longevity							
Linear	365.78	356.48	2	432	5.64	0.004^E	14.3 (6.7, 25.4)
Quadratic	312.97	311.72	2	428	0.86	0.42	
Correlational	311.72	311.02	1	426	0.96	0.33	
B. Females							
Development time vs. Body size							
Linear	287.49	253.67	2	446	29.73	0.0001^F	42.6 (36.1, 49.1)
Quadratic	205.49	176.37	2	442	36.49	0.0001^G	
Correlational	174.83	174.72	1	440	0.27	0.60	
Development time vs. Longevity							
Linear	351.78	298.86	2	446	39.49	0.0001^H	56.3 (44.8, 68.7)
Quadratic	282.58	249.90	2	442	28.90	0.0001^I	
Correlational	245.85	233.05	1	440	24.17	0.0001	
Body size vs. Longevity							
Linear	374.43	366.17	2	446	5.03	0.007^J	15.9 (7.5, 26.9)
Quadratic	326.61	317.70	2	442	6.20	0.002^K	
Correlational	312.23	301.66	1	440	15.41	0.0001	

Univariate: ^A P: $F_{1,432} = 15.02, P = 0.0001$; C: $F_{1,432} = 3.09, P = 0.08$; ^B P: $F_{1,428} = 49.57, P = 0.0001$; C: $F_{1,428} = 35.61, P = 0.0001$; ^C P: $F_{1,432} = 35.95, P = 0.0001$; C: $F_{1,432} = 26.54, P = 0.0001$; ^D P: $F_{1,428} = 55.69, P = 0.0001$; C: $F_{1,428} = 23.36, P = 0.0001$; ^E P: $F_{1,432} = 5.31, P = 0.02$; C: $F_{1,432} = 11.17, P = 0.0009$; ^F P: $F_{1,446} = 55.57, P = 0.0001$; C: $F_{1,446} = 17.70, P = 0.0001$; ^G P: $F_{1,442} = 34.90, P = 0.0001$; C: $F_{1,442} = 54.48, P = 0.0001$; ^H P: $F_{1,446} = 77.75, P = 0.0001$; C: $F_{1,446} = 53.01, P = 0.0001$; ^I P: $F_{1,442} = 53.79, P = 0.0001$; C: $F_{1,442} = 13.40, P = 0.0003$; ^J P: $F_{1,446} = 3.10, P = 0.08$; C: $F_{1,446} = 9.46, P = 0.0022$; ^K P: $F_{1,442} = 4.42, P = 0.036$; C: $F_{1,442} = 5.08, P = 0.025$.

6.4.4 Fitness within the sexes

6.4.4.i Males

Predicted values for fitness were calculated using optimal values from the effects of P and C content on shared life-history traits; development time, longevity

and body size (see Lewis *et al.*, 2011 for method). Consequently, it was found that the content of both P and C in the diet had significant linear effects on the predicted fitness of males (Table 6.3 and Figure 6.2a). Higher content of both nutrients increased predicted male fitness i.e. male mating success. There was a significant quadratic effect of C content, causing a peak in predicted male fitness (Table 6.3). Inspection of the nutritional landscape showed that higher predicted fitness for males occurred on diets with an approximate equal ratio of P:C (Figure 6.3a). There were no significant quadratic effects of P content, as well as no significant correlational gradient (Table 6.3).

Realized fitness values from a subset of male moths reared on the diets were obtained. C content was found to have significant linear and nonlinear effects on male mating success (Table 6.3; Figure 6.3c), which are similar to the findings for male predicted fitness. There were no significant effects of P content on male realized fitness, nor correlational effects of the two macronutrients (Table 6.3). Inspection of the nutritional landscape revealed a peak for male mating success, at an approximate 1:1 ratio of P:C (Figure 6.3c).

A formal comparison of predicted and realized fitness landscapes for males was conducted, in order to compare the effects of P and C between the two fitness measures. P and C were found to differ in their linearity between the two landscapes ($F_{2,373} = 13.57, P = 0.0001$). The significant difference in linearity was due to predicted fitness being more responsive to P ($F_{1,373} = 24.50, P = 0.0001$) and C content ($F_{1,373} = 22.72, P = 0.0001$) in the diets than realized fitness in males (Table 6.3). There were marginally non-significant quadratic ($F_{2,369} = 2.37, P = 0.09$) and correlational effects ($F_{1,367} = 3.10, P = 0.08$) of P and C between the two landscapes. Overall, a comparison of the nutritional landscape for male predicted (Figure 6.3a) and realized (Figure 6.3c)

fitness look remarkably similar, with the angle between the two linear vectors being moderately large ($\vartheta = 35.9^\circ$, 95% CI: 20.8° , 48.5°). Peak fitness for both realized and predicted fitness in males occurs in approximately the same region of the landscape, but stronger responses were generated for predicted fitness compared to realized fitness in male moths.

6.4.4.ii Females

Similarly to males, predicted values for fitness were calculated for females. Content of P and C in the diet both had a significant negative linear effect on predicted female fitness (Table 6.3). Lower content of P and C in the diet caused higher female fitness i.e. lifetime fecundity. There were significant quadratic effects of P and C content, indicating a peak in predicted fitness values (Table 6.3). Inspection of the nutritional landscape showed the peak to be around a 1:2 ratio of P:C (Figure 6.3b). There was a significant negative correlational gradient for the effect of P and C on predicted fitness (Table 6.3), which corroborates that lower P and C content in the diet led to higher predicted fitness in females.

We used actual fitness values from female moths reared on the differing diets, in order to determine the realized effects of P and C content on female lifetime fecundity. Both P and C content had significant negatively linear effects on realized female fitness (Table 6.3; Figure 6.3d), which corroborates the findings from predicted fitness for females. Lower content of P and C in the diet caused higher female fecundity. There was no quadratic effect of C content, and a marginally non-significant quadratic effect of P content (Table 6.3). Visual inspection of the nutritional landscape showed that although no significant peaks were observed, higher female fitness

tended towards diets with a 1:2 ratio of P:C (Figure 6.3d). There was no correlational effect of dietary P and C on realized female fitness (Table 6.3).

The nutritional landscapes for predicted and realized fitness in females were remarkably similar, and in order to compare the effects of dietary P and C between the two fitness measures a statistical analysis using a sequential model building approach was used. P and C were found to differ significantly in linear ($F_{2,368} = 18.78, P = 0.0001$), quadratic ($F_{2,364} = 11.25, P = 0.0001$), and correlational ($F_{1,362} = 7.80, P = 0.006$) effects. The significant difference in linearity was due to predicted fitness values being more responsive to both P ($F_{1,368} = 20.01, P = 0.0001$), and C ($F_{1,368} = 37.44, P = 0.0001$) content in the diet than realized fitness in females (Table 6.3). Significant quadratic differences were due to P content ($F_{1,364} = 19.72, P = 0.0001$) causing a peak in predicted female fitness, as evidenced through a negative coefficient gradient, as opposed to a marginally non-significant effect of P content on realized fitness in females (Table 6.3). There was no significant difference in the quadratic effect of C content ($F_{1,364} = 0.01, P = 0.91$) between predicted and realized fitness in females. The significant difference in correlational effect was due to a significant negative correlation of P and C content on predicted fitness, compared to a non-significant correlation on realized fitness for females (Table 6.3). Overall, the nutritional landscapes for predicted (Figure 6.3b) and realized (Figure 6.3d) fitness look fairly similar. The angle between the two linear vectors for peak fitness is relatively large ($\vartheta = 43.8^\circ, 95\% \text{ CI: } 25.4^\circ, 68^\circ$), indicating that peaks for predicted and realized females occur in slightly different spaces of the geometric framework, due to stronger responses being generated for predicted fitness over realized fitness in female moths.

Table 6.3. The linear and nonlinear effects of protein (P) and carbohydrate (C) content on predicted and realized fitness in (A) male and (B) female *Plodia interpunctella*. Significant P values are highlighted in **bold**.

Response variables	Linear effects		Nonlinear effects		
	P	C	P x P	C x C	P x C
(A) Males					
<i>Predicted fitness</i>					
Coefficient ± SE	0.66 ± 0.08	0.87 ± 0.08	-0.04 ± 0.11	-0.24 ± 0.10	0.22 ± 0.19
t_{218}	8.50	11.55	0.33	2.41	1.14
<i>P</i>	0.0001	0.0001	0.74	0.017	0.26
<i>Realized fitness</i>					
Coefficient ± SE	0.03 ± 0.19	0.48 ± 0.18	-0.23 ± 0.27	-0.56 ± 0.27	-0.63 ± 0.49
t_{159}	0.17	2.70	0.87	2.04	1.28
<i>P</i>	0.86	0.008	0.39	0.04	0.20
(B) Females					
<i>Predicted fitness</i>					
Coefficient ± SE	-0.94 ± 0.06	-1.03 ± 0.06	-0.38 ± 0.07	-0.22 ± 0.07	-0.34 ± 0.13
t_{225}	14.78	16.78	5.40	3.20	2.65
<i>P</i>	0.0001	0.0001	0.0001	0.002	0.009
<i>Realized fitness</i>					
Coefficient ± SE	-0.37 ± 0.13	-0.27 ± 0.12	0.31 ± 0.18	0.17 ± 0.20	0.54 ± 0.34
t_{147}	2.91	2.26	1.73	0.87	1.57
<i>P</i>	0.004	0.025	0.09	0.38	0.12

6.4.5 Shared life-history traits between the sexes

A sequential model building approach was used to compare the linear, quadratic and correlational effects of P and C content on the three shared life-history traits (development time, longevity and body size) between the sexes. There was a significant linear difference in effect of P ($F_{1,439} = 5.60$, $P = 0.02$; Table 6.4) content on body size between the sexes, but not in C content ($F_{1,439} = 1.29$, $P = 0.26$; Table 6.4), indicating that P content generated a larger response in male, but not female body size (Table 6.1). There were no significant quadratic or correlational differences between the sexes for body size (Table 6.4). Inspection of the nutrient landscapes for male (Figure 6.2c) and female (Figure 6.2d) body size shows that the optimal nutrient

content occurred in the same region, as shown by a relatively smaller angle ($\vartheta = 17.2^\circ$; Table 6.4) between the linear vectors for body size.

There were no significant linear or quadratic effects of P or C content on lifespan between the sexes (Table 6.4). A significant correlational effect was observed for longevity (Table 6.4). This was due to a stronger gradient for P x C effects on female lifespan compared to males (Table 6.1). A comparison of the nutrient landscapes for longevity between males (Figure 6.2e) and females (Figure 6.2f) revealed that optimal lifespan has diverged between the sexes, as illustrated by the moderate angle between them ($\vartheta = 26.2^\circ$; Table 6.4).

There were no significant differences in the linear, quadratic or correlational effects of P or C content on development time between the sexes (Table 6.4). Comparison of the nutrient landscapes for males (Figure 6.2a) and females (Figure 6.2b) showed that shorter development times occurs in the same region for both sexes, as evidenced by the small angle between the two linear vectors ($\vartheta = 6.1^\circ$; Table 6.4).

In summary, both sexes both achieve shorter development times on equal ratios of P:C in their diet. Larger body size also peaks in approximately the same nutrient space for males and females, at a ratio of 1:2 P:C. In contrast, we see a shift in the peaks for optimal male and female lifespan, with C content generating a stronger response in females compared to males.

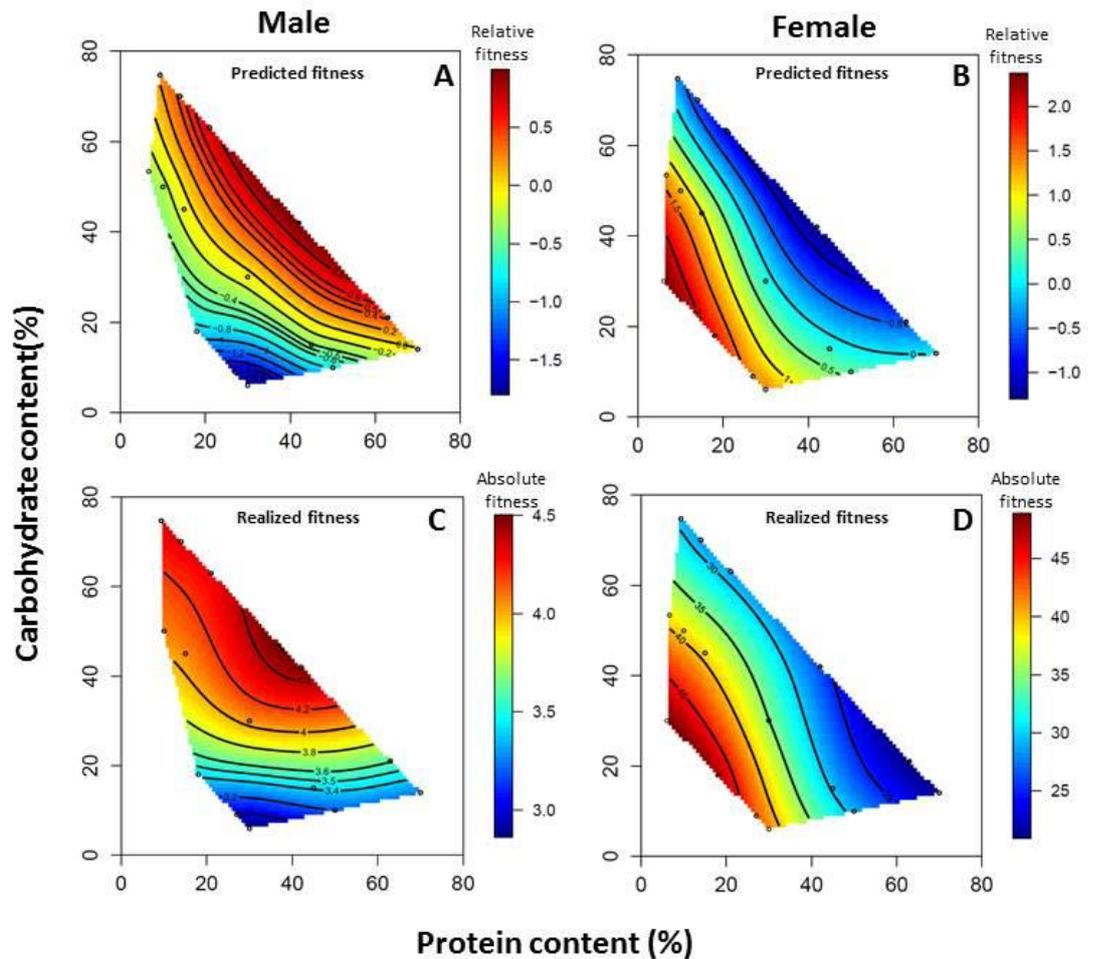


Figure 6.3: The nutritional landscapes for male predicted fitness (a), male realized fitness (c), female predicted fitness (b), and female realized fitness (d). In each landscape the red regions represent higher values for fitness, whilst the blue regions represent lower values for fitness.

6.4.6 Fitness between the sexes

There were significant differences in linear, nonlinear and correlational effects of P and C on predicted fitness between the sexes (Table 6.4). The significant difference in linear effects between males and females was due to the opposing predicted fitness effects of both P ($F_{1,439} = 255.87, P = 0.0001$; Table 6.3) and C content in the diets ($F_{1,439} = 385.13, P = 0.0001$; Table 6.3). Both P and C content show

significant positive linear gradients for predicted fitness in males, in contrast to the significant negative gradients for predicted fitness in females (Table 6.3). There were significantly different quadratic effects of both P ($F_{1,435} = 75.44, P = 0.0001$; Table 6.4) and C content ($F_{1,435} = 12.85, P = 0.0004$; Table 6.4) on predicted fitness between males and females. This was due to very significant peaks for both P and C content in females for predicted fitness, whereas a significant peak for males was only observed for C x C effects (Table 6.3). There was a significant correlational effect of P and C content on predicted fitness between the sexes (Table 6.3), due to opposing correlational gradients being observed for P x C between the sexes (Table 6.3). Comparison of the nutrient landscapes for predicted male (Figure 6.3a) and predicted female (Figure 6.3b) fitness shows that optimum fitness is achieved in opposing regions, as indicated by the extremely large angle of difference between the two linear vectors ($\vartheta = 165.5^\circ$; Table 6.4).

Linear and correlational effects of P and C were found to be significant on realized fitness between the sexes (Table 6.4). The significant difference in linear effects were due to the opposing realized fitness effects of both P ($F_{1,302} = 5.40, P = 0.02$; Table 6.3) and C ($F_{1,302} = 12.10, P = 0.0006$; Table 6.3) content in the diets. Both P and C content show significant positive gradients for male, in contrast to negative gradients in females (Table 6.3). There was a significant correlational effect of P and C on realized fitness between the sexes due to opposing gradients for P x C between the sexes (Table 6.3). There were no significant quadratic effects of P and C on realized fitness between the sexes (Table 6.4). Visual inspection of the nutrient landscape for realized male (Figure 6.3c) and realized female (Figure 6.3d) fitness shows once again that optimum fitness is achieved in opposing regions, as indicated by the orthogonal angle between the two linear vectors ($\vartheta = 85.77^\circ$; Table 6.4).

In summary, a higher content of P and C at an 1:1 ratio in the diet led to increased predicted and realized male fitness, whilst a negative relationship was found for females, with lower P:C content increasing predicted and realized female fitness. Both predicted and realized fitness of males and females was achieved in opposing regions of the nutrient landscape, indicative of sexual antagonism for fitness over dietary P and C content.

Table 6.4. Sequential model based approach comparing the linear, quadratic and correlational effects of protein (P) and carbohydrate (C) content on shared life-history traits (development time, longevity and body size), and fitness between the sexes. Significant *P* values are highlighted in **bold**.

	<i>SS_R</i>	<i>SS_C</i>	<i>DF₁</i>	<i>DF₂</i>	<i>F</i>	<i>P</i>	<i>θ</i> (95%CI)
Development time							
Linear	208.30	207.60	2	439	0.74	0.48	6.1 (2.9, 10.4)
Quadratic	126.72	126.67	2	435	0.09	0.92	
Correlational	121.12	120.36	1	433	2.73	0.10	
Body size							
Linear	318.14	313.50	2	439	3.25	0.04^A	17.2 (9.6, 24.8)
Quadratic	251.82	250.33	2	435	1.29	0.28	
Correlational	249.50	249.49	1	433	0.00	0.98	
Longevity							
Linear	411.81	409.14	2	439	1.43	0.24	26.2 (13.3, 41.8)
Quadratic	380.09	379.10	2	435	0.57	0.56	
Correlational	368.61	363.18	1	433	6.47	0.011	
Predicted fitness							
Linear	437.34	231.83	2	439	194.58	0.0001^B	165.5 (160, 180)
Quadratic	213.05	170.38	2	435	54.47	0.0001^C	
Correlational	170.21	167.88	1	433	6.00	0.015	
Realized fitness							
Linear	296.06	284.40	2	302	6.19	0.002^D	85.8 (60.7, 111.8)
Quadratic	281.40	280.75	2	298	0.34	0.71	
Correlational	280.74	276.89	1	296	4.12	0.04	

Univariate: ^A P: $F_{1,439} = 5.60$, $P = 0.02$; C: $F_{1,439} = 1.29$, $P = 0.26$; ^B P: $F_{1,439} = 255.87$, $P = 0.0001$; C: $F_{1,439} = 385.13$, $P = 0.0001$; ^C P: $F_{1,435} = 75.44$, $P = 0.0001$; C: $F_{1,435} = 12.85$, $P = 0.0004$; ^D P: $F_{1,302} = 5.40$, $P = 0.02$; C: $F_{1,302} = 12.10$, $P = 0.0006$

6.5 Discussion

Application of the geometric framework allowed us to examine the effect of protein (P) and carbohydrate (C) consumption on a number of traits in *Plodia interpunctella*. Survival of larvae to adult moths was optimised on diets containing a 1:2 ratio of P:C (see Figure 6.1). Of the surviving adults, three shared life-history traits (development time, longevity and body size) were measured within and between the sexes. Both males and females optimised their development time (see Figures 6.2a and b), and body size (see Figures 6.2c and d) in approximately the same nutrient space, with a 1:1 ratio and 1:2 ratio of P:C respectively. A shift in peaks for lifespan between the sexes was observed, as C content had a stronger response on female lifespan compared to males, which was corroborated by a 1:5 ratio of P:C for females, compared to a 1:2 ratio of P:C for males (see Figures 6.2e and f). Finally, the impact of diet on male and female fitness was determined both through predicted values, as well as using experimental moths reared on the various diets. Peaks for male and female fitness were shown to be in diametrically opposing regions for both predicted (see Figures 7.3a and b), and realized fitness (see Figures 6.3c and d). Sexual antagonism is therefore not resolved through dietary protein and carbohydrate content in *P. interpunctella*.

6.5.1 Survival

The content of both P and C in the diet had significant multivariate effects on the proportion of larvae surviving until eclosion as adult moths. Higher proportion of larvae were found to survive on diets with a higher content of P and C. Carbohydrate content generated a stronger response in survival, with a peak observed at a ratio of 1:2 P:C in the nutritional landscape. The finding that larval survival was higher on diets

with more carbohydrates than protein is consistent with the findings of a similar study in another capital breeding moth, *Heliothis virescens* (Roeder and Behmer, 2014). Alongside Roeder and Behmer (2014), our study is one of a few that specifically examine the effects of larval diets in adult Lepidopterans. The pupal stage is energetically very costly to holometabolous species (Boggs, 2009), such as *P. interpunctella*, that favour higher carbohydrate content in diets to provide metabolic fuel. As life-history traits and fitness could only be measured in moths that survived developing larvae were subject to strong selection. Through the use of 24 artificial diets in the geometric framework, we have identified diets that were optimal for survival, as well as those that were not, due to lower nutritional content. This approach differs from previous studies on Lepidopteran larvae that used a maximum of 7 diets (Telang et al., 2001; 2003; Lee, 2010; Roeder and Behmer, 2014). Whilst we recorded total proportion of larvae surviving, we were unable to determine the sex of larvae when we placed them on diets as first instars as the pigmented testes in males are not visible at this stage (Ingleby *et al.*, 2010). Hence, whether there are sex-specific mortality effects of dietary P and C remains to be examined.

6.5.2 Shared life-history traits

Of the surviving larvae, sex-specific effects of P and C were determined for three shared life-history traits (development time, longevity and body size; Lewis *et al.*, 2011). Multivariate selection on development time in both males and females was found to be positive and significant, indicating that both nutrients were important to development times. An equal balance of increasing protein and carbohydrate content led to a high degree of similarity in the nutritional landscapes between males and females for development time, as evident through their respective peaks at a 1:1 ratio

of P:C. An equal ratio of protein and carbohydrate is beneficial in this capital breeding species of moth, as all energy required for reproduction and metabolically costly stages during development (such as pupation) is acquired during larval feeding. Our findings of equal ratios of dietary P and C leading to shorter development times are corroborated by similar findings in the capital breeding moth *H. virescens* (Roeder and Behmer, 2014). Development time is predicted to be shorter in male *P. interpunctella* in order to increase their reproductive success, as it provides more opportunity to find females and secure copulations (Lewis, 2005; Lewis *et al.*, 2011). In contrast, female *P. interpunctella* are predicted to favour longer development times (Lewis *et al.*, 2011), as this allows more time for resources to be gained through larval feeding as the nutritional content of their natural diets are a poorer resource of protein. This opposing linear selection acting on development times in the sexes may be the result of sexual antagonism.

Body size for both sexes increased with increasing P and C in the diet, but carbohydrate content was increasingly more important to body size. Maximal body sizes were achieved at a 1:2 ratio of P:C, indicating that content of P and C needed to be balanced for male and female *P. interpunctella*. Few studies have measured body size *per se*, but have instead measured body mass (Gall and Behmer, 2014), or dried pupal mass (Roeder and Behmer, 2014), corroborating our findings that a carbohydrate biased diet is important for growth. Although body size in male *P. interpunctella* has been shown to have no significant impact on mating success in male-male competition (Cook *et al.*, 1997; Chapter 2), there may be other factors that favour larger male size, such as bigger males may be better at courting/ seducing females, or preferred by females in order to obtain bigger nutritious spermatophores (Fritzsche and Arnqvist, 2015). However, female fecundity or lifespan benefits derived

from male spermatophores are absent in *P. interpunctella* (Cook, 1999). Larger female body size is positively correlated with fecundity in many insects (Honěk, 1993; Blanckenhorn, 2000), which is also corroborated in *P. interpunctella* (Lewis *et al.*, 2011; Chapter 2). Larger body size is suggested to either reflect more resources accrued in the larval stage for egg production (García-Barros, 2000), or required to accommodate increasing numbers of eggs (Preziosi *et al.*, 1996, and Winkler *et al.*, 2012).

Lifespan was the only shared life-history trait that showed sex-specific divergence in response to dietary protein and carbohydrate. Carbohydrate was the key nutrient in both sexes associated with longer lifespan, yet whilst males optimised lifespan on a 1:2 ratio of P:C, females required a low P:C ratio of 1:5 in order to maximise their longevity. Dietary restriction has been shown in a number of taxa to increase lifespan, with a more pronounced effect in females (Nakagawa *et al.*, 2012), as they appear to invest more in somatic maintenance leading to reduced fecundity (Partridge *et al.*, 2005). In Female *P. interpunctella* lifespan was reduced when diets become more protein biased. This result mirrors previous findings showing that lifespan extension is achieved under a restriction of certain nutrients i.e. protein, and not due to caloric restriction as in *Drosophila melanogaster* for example (Lee *et al.*, 2008; Maklakov *et al.*, 2008; Jensen *et al.*, 2015). This negative effect on lifespan of higher protein content was more pronounced in female *P. interpunctella* than males, and may be driven by sex-differences in reproductive effort (Bonduriansky *et al.*, 2008). Our results indicate that the content of protein and carbohydrate in the diet led to sex-specific effects in *P. interpunctella*, with consumption of carbohydrate being key for lifespan extension.

6.5.3 Life-history trade-offs

Shared life-history traits were compared within the sexes, revealing different trade-offs resulting from dietary P and C content. Increased dietary protein favoured shorter development times in both sexes, at 1:1 P:C ratio. In contrast, carbohydrates were favoured for both body size and lifespan. Diets high in protein seem to drive trade-offs, as a comparison of nutrient landscapes revealed divergence between development times (favouring high P) compared to the lifespan and body size landscapes (Table 6.2). Larger differences between nutritional landscapes were evident for females, especially for development time and lifespan. Longer development times can be indicative of key nutrients being deficient in the diet, such as nitrogen (Tigreros, 2013) and protein (Edgar, 2006; in contrast see Jaramillo *et al.*, 2015). However, our results indicate that increased protein content favours shorter development time over lifespan and body size, especially for female *P. interpunctella*.

No apparent trade-offs were found between body size and lifespan over nutritional content of diets within both sexes. Longevity and body size had very similar nutritional landscapes, indicating that a more carbohydrate-biased diet was favourable for both traits. The manipulation of nutritional composition of diets has been suggested as a method for detecting trade-offs in a variety of traits within species (Raubenheimer and Simpson, 2007). Life-history trade-offs are ubiquitous (Zera and Hershman, 2001), yet rarely studied within the geometric framework (Clark *et al.*, 2013, 2015).

6.5.4 Fitness

Lifetime fitness was compared between the sexes through quantifying multivariate selection on the three shared life-history traits relating to fitness (Lewis *et*

al., 2011). Importantly, we found that male and female fitness peaked in diametrically opposing regions of the nutrient landscape. Protein and carbohydrate content in diets were selected in opposite directions as indicated by the response surfaces of males and females. Males increased their reproductive success at a higher content of both nutrients, with peak at an equal ratio of P:C. In contrast, predicted female fitness was negatively associated with protein and carbohydrate content, with lower contents of P and C leading to a peak in predicted female fecundity at a P:C ratio of 1:2. The findings for predicted fitness are indicative of sexual antagonism for fitness over dietary P and C content. This corroborates the findings of Lewis *et al.* (2011a), where *P. interpunctella* was shown to be under strong intralocus sexual conflict due to opposing selection for shared fitness-related life-history traits.

Furthermore, observed fitness values were obtained from a subset of adult moths that survived and eclosed on the varying artificial diets. A comparison between the sexes again revealed that dietary P and C were selected in opposing regions of the nutrient landscape (see Figure 6.3c and d). Male fitness was maximised on diet containing a relatively high content of P and C at an equal ratio, in a region of the nutritional landscape that corresponds to optimal male development time, indicating both nutrients are key for male reproductive success. As males face competition for mates, and court females, they require high contents of nutrients to fuel these metabolically costly behaviours. Carbohydrates are known to provide a rich source of energy for males (Maklakov *et al.*, 2008; South *et al.*, 2011), and can be used in advertisement calling (Hunt *et al.*, 2004). In the cockroach *Nauphoeta cinerea* sperm number and male fertility were found to be optimal at 1:2 ratio P:C (Bunning *et al.*, 2015), indicating that higher contents of protein and carbohydrates are important to male reproductive success, supporting our findings here. Roeder and Behmer (2014)

determined egg viability as a measure of mating success for male *H. virescens* moths, and found it is optimised on diets of approximately equal ratios of P and C. However, our study is the first one to determine male Lepidopteran fitness in terms of how many successful copulations males obtain over a lifetime. Determining male mating success is especially important in *P. interpunctella*, as even if a male develops on poor larval diet, he still transfers the same amount of sperm per mating, but may not be able to mate as often (Lewis *et al.*, 2011b).

Female observed fitness showed a negative linear relationship with dietary protein and carbohydrates. Lower contents of P and C led to higher female lifetime fecundity, in a similar region of the nutrient landscape that favoured longer development time. Lifetime fecundity was also maximised in an opposing region of the nutrient landscape compared to increased lifespan, another life-history trait known to trade-off with reproductive success (Chapman *et al.*, 1998). Longer development times are known to be associated with increased female fitness in *P. interpunctella* (Lewis *et al.*, 2011). Our finding of higher fitness associated with lower content of P and C in female *P. interpunctella* is in contrast to other insect species such as the field cricket *Teleogryllus commodus* (Maklakov *et al.*, 2008), the fruit fly *D. melanogaster* (Lee *et al.*, 2008), and Queensland fruit flies (Fanson *et al.*, 2009; Fanson and Taylor, 2012), which are all income breeders and able to supplement their diet through adult feeding (Wheeler, 1996). Perhaps *P. interpunctella* have adapted to efficiently convert the limited protein availability in their larval diet into nutrient rich processes, such as oogenesis. For example, female larvae of the Oriental leafworm moth, *Spodoptera litura* convert protein more efficiently than males needed for body growth requirements (Lee, 2010). Females may also ingest more of the diets in order to provide the required amounts of nutrients beneficial to increased fecundity (Telang,

2001; Lee, 2010). However, we did not directly measure food intake in either of the sexes, as *P. interpunctella* live in and amongst their larval medium, making this very challenging.

Although observed fitness between the sexes in their nutrient landscapes is opposing, there was a greater divergence for predicted fitness. The differences between the two fitness landscapes between the sexes are likely driven by genetic constraints due to shared life-history traits preventing either sex to reach their sex-specific optima over diet choice. Shared life-history traits have a common genetic basis in the sexes, yet have sex-specific optima (Lewis *et al.*, 2011; Chapter 6). Observed fitness may be constrained over macronutrient content, leading to a smaller divergence of nutrient landscapes compared to predicted fitness, as carbohydrate was the only nutrient shown to have a significant effect. *P. interpunctella* are pests of stored grains (Williams, 1964), which are naturally high in carbohydrates and low in protein. As the larvae have not evolved with excess protein in their diet, *P. interpunctella* may not be adapted to processing the amounts of dietary protein supplied through the geometric framework. Previous research has proposed the lethal protein hypothesis (Simpson and Raubenheimer, 2007, 2009), suggesting that high intakes of protein may be detrimental, as animals cannot readily digest it.

Comparison of the nutrient landscapes within the sexes generated for both predicted and observed fitness showed that the peaks for respective reproductive success were in similar regions for males and females (Figure 6.3). This indicates that predicted and observed fitness had similar dietary requirements of protein and carbohydrate, demonstrating the robustness of our findings for *P. interpunctella*. The smaller sample size used for observed fitness is likely to play a role in the differences between fitness landscapes within the sexes, as logistically we were not able to record

fitness in every animal that eclosed on the artificial diets. However, the difference between the two nutritional landscapes for fitness are more likely to be driven by genetic constraints between the sexes, as well rearing moths on diets that contained macronutrients (protein) not readily available in their natural diets.

6.5.5 Conclusion

Few studies have employed nutritional geometry as a method to elucidate the effects of dietary protein and carbohydrate in males and females. Conflicts between the sexes are often present as their reproductive ideals rarely align (Maklakov *et al.*, 2008; Reddiex *et al.*, 2013; Jensen *et al.*, 2015). Capital breeding species such as Lepidopterans have largely been ignored, certainly past the pupal stage in the geometric framework (Telang *et al.*, 2001; 2003; Lee, 2010; Roeder and Behmer, 2014). Importantly, we found that sex-specific effects of diets on reproductive success were in opposed regions of the nutrient landscape for *P. interpunctella*. Genetic correlations between the sexes exist over shared life-history traits, resulting in a tug-of-war over respective optima for reproductive success. Here we demonstrate that despite a vast complement of diets spanning the whole nutrient landscape, intralocus conflict is not resolved. Carbohydrate content in the diet was found to have a key role in affecting observed fitness, likely indicative of the ecology of *P. interpunctella* as it is a pest species living in stored products that are high in carbohydrates. Our study clearly highlights the importance of using a geometric framework approach to fully understand the effects of nutritional components of diet over shared life-history traits and fitness in both sexes.

7. General Discussion.

7.1 Introduction

This thesis investigates sexual conflict and sexual selection in the Indian meal moth, *Plodia interpunctella*. Altering the adult mating sex ratio by experimental evolution to male biased (MB) or female biased (FB) changed the selective forces between populations that may have impacted on intralocus sexual conflict. Overall, both sexes in the MB populations had increased reproductive success with corresponding changes in shared life-history traits compared to FB populations (Chapter 2). MB populations also had higher immune response function when dosed with a natural viral pathogen (Chapter 4), further supporting divergence between the two treatments. MB males were subject to increased selection on traits to defend their sperm from a rival male's ejaculate (Chapter 3). Despite these differing reproductive tactics, overall offspring sired was equal by males from either sex-ratio treatment. Fecundity selection was examined in stock lines of *P. interpunctella*, and after six of generations of selection lifetime fecundity was increased, without corresponding changes or trade-offs with body size and longevity (Chapter 5). Finally, the effect of the macronutrients protein (P) and carbohydrate (C) was examined in a stock population using the geometric framework (Chapter 6). We found that sexually antagonistic selection over diet choice was evident, with males and females showing preference for P and C in opposing regions of the nutrient landscape, indicating that intralocus sexual conflict is unlikely to have been resolved in this moth species.

7.2 Pre- and Post-Copulatory Selection

Selection for increased reproductive success in the sexes can occur both pre- and post-copulatory, resulting in evolution of traits that confer an advantage in competitive environments (Darwin, 1871; Andersson, 1994). By altering the levels of conflict faced under biased adult mating sex ratios, populations diverged in response to this selection. We aimed to create environments that would favour the more numerous sex as a way of altering sexual conflict levels since they would be subject to more intense selection (Kokko and Jennions, 2008). Males in the MB responded as we expected, by securing more successful copulations over their lifetime (Chapter 2). It appears that pre-copulatory selection is of more importance in this species, corroborating previous research (Bateman, 1948; Andersson and Simmons, 2006). Whilst males in the MB and FB adopt two very different strategies regarding sperm competitive ability, they sire the same number of offspring overall (Chapter 3). As we have shown, if MB males secure one extra copulation, this should increase their overall reproductive success and allow them to sire more offspring

Furthermore, we found evidence of divergence in shared life-history traits that relate to increased male reproductive success. Males in MB populations developed quicker, had longer lifespans and larger body sizes in comparison to males from FB populations (Chapter 2). A recent study in the laboratory conducted competitive matings under the same conditions the moths have been evolving in. Twelve males and 4 female moths were placed together upon eclosion to represent the MB adult mating sex ratio (3:1 M:F) and male mating success was quantified. Overall, there was a 17% chance of a male securing a mating under these competitive conditions indicating there is a large variance in male mating success in the MB treatment.

Successful males (securing at least one mating) were also found to develop faster ($F_{1,669} = 8.1$, $P < 0.004$), and had a marginally significant trend to live longer ($F_{1,626} = 3.25$, $P = 0.06$) (Larner *et al.*, unpublished data). Body size had no significant effect on the likelihood of achieving successful copulations in a competitive mating environment ($F_{1,588} = 0.39$, $P = 0.56$). This corroborates previous findings that shared life-history traits are correlated to fitness (Lewis *et al.*, 2011), and males that develop faster and live longer should have increased reproductive success (Chapter 2).

7.3 Resolution of IntraLocus Conflict

Recent research has focussed on quantifying intralocus conflict (IASC) in a number of species (e.g. fruit flies, Chippindale *et al.*, 2001; ground cricket, Fedorka and Mousseau, 2004; fruit flies, Pischedda and Chippindale, 2006; red deer, Foerster *et al.*, 2007; Indian meal moth, Lewis *et al.*, 2011). However, it remains to be established if IASC can be resolved within a species (Bonduriansky and Chenoweth, 2009; Cox and Calsbeek, 2009; Pennell and Morrow, 2013). We found that sex-specific effects of diets on reproductive success were in opposed regions of the nutrient landscape for a stock population of *P. interpunctella*, indicating intralocus conflict was not resolved despite the nutrient space available (Chapter 6). This finding corroborates previous research on the presence of IASC, indicating the robustness and strength of sexual conflict in this species (Lewis *et al.*, 2011).

However, altering the adult mating sex ratio can impact mating behaviours, strategies and reproductive success (Weir *et al.*, 2011; Chapter 2). Male-biased ratios will increase male-male competition, as females are in limited supply, consequently affecting sexual selection on the sexes, and potentially altering their evolutionary

trajectory (Bonduriansky and Chenoweth, 2009). We found that both sexes in MB populations diverge towards their respective sex-specific fitness optima. Increased fitness and correlated changes in shared life-history traits suggest that conflict resolution may be under way in the MB populations as both males and females had higher reproductive success (Chapter 2). Sexual selection is also likely to be a potent force driving these results, as increased competition for mates drives higher male mating success, increased fecundity in females, and increased hatching rates of eggs within the MB populations. To potentially quantify if conflict resolution has occurred in these diverged experimental evolution lines, sex-biased gene expression could be examined within the sexes. It has been suggested that sex-biased gene expression may be able to ameliorate sexual conflict between the sexes (Mank *et al.*, 2011).

7.4 Trade-Offs

7.4.1 Life-history traits

Life-history traits are often traded-off against reproductive success, as resources are allocated towards fecundity in females or mating success in males over somatic maintenance (Zera and Hershman, 2001; Partridge *et al.*, 2005). In Chapters 2, 5, and 6 we found that as female fecundity increased, there was a consistent negative correlation with lifespan, corroborating findings from other insect species (Hönek, 1993; Chapman *et al.*, 1998; Partridge *et al.*, 2005), as well as previous research in *P. interpunctella* (Lewis *et al.*, 2011). Yet, despite significantly increasing lifetime fecundity in *Fecundity Selected* females, we found no corresponding changes in lifespan or body size, indicating direct selection on fecundity occurred without affecting the trade-off with longevity, possibly indicating that the two traits may be

uncoupled (Chapter 5). As a corollary, the lack of differences in lifespan between *Fecundity Selected* and *Control* lines may represent a shift in egg-laying patterns. Although overall lifetime fecundity was increased in *Fecundity Selected* lines, early fecundity was higher in *Control* lines, suggesting that females may employ different egg laying strategies in order to maximise their reproductive fitness. If differing strategies are employed, it may be harder to observe trade-off between fecundity and life-span.

7.4.2 Immunity

MB and FB lines showed evolved divergence in protection from a viral pathogen (Chapter 4). FB lines had higher proportions of larvae showing signs of infection compared to MB populations, likely driven by previous findings that FB populations have lower circulating phenoloxidase levels (McNamara *et al.*, 2013b). Trade-offs may be mediating the divergence in viral susceptibility, as males from FB invest more into higher mating frequencies and copulation (Ingleby *et al.*, 2010), possibly at the expense of reduced protection from a viral pathogen. Although we could not measure sex-specific effects of immunity due to using third instar larvae, research has shown that as males invest more into securing matings there may be a lowered immune function compared to females (McKean and Nunney, 2001; Rolff, 2002; McNamara *et al.*, 2013a). Potential research investigating this possibility using our diverged experimental evolution lines by allowing them to evolve in the presence of sub-lethal doses of *Plodia interpunctella* granulosis virus (*PiGV*) is needed to address this question. Sex-specific measures of immune response could then be investigated, using phenoloxidase titres, as well as determining viral loads within males and females.

7.4.3 Diet

Trade-offs over dietary protein and carbohydrate in *P. interpunctella* were evident within the sexes (Chapter 6). Increased protein in the diet favoured shorter development times in both sexes, and it was found that body size and lifespan extension were achieved on more carbohydrate biased diets. A stronger trade-off was generated in female moths, as a comparison between nutrient landscapes generated for each life-history trait indicated a greater degree of divergence between development time vs. body size and/ or longevity. Few studies have used the geometric framework to investigate physiological trade-offs (Lee *et al.*, 2008; Maklakov *et al.*, 2008; Clark *et al.*, 2015), as well as trade-offs over diet and immune function (Cotter *et al.*, 2011). This highlights the importance of using such nutritionally complex diets to elucidate the overall condition and allocation of resources available within species in order to favour traits that confer high fecundity or mating success advantages.

7.5 Ecology of *Plodia interpunctella*

P. interpunctella are common pests, with larvae causing loss of food as they live in and amongst stored grains (Williams, 1964; Mohandass *et al.*, 2007). As a pest species, they are likely selected to maximise their reproductive output, as we found in Chapter 5. After targeting selection on female fecundity we found a small, but significant increase in lifetime fecundity in comparison to control lines. However, total eggs produced did not differ significantly from start of the experiment until its cessation (Figure 5.2). This suggests that when conditions are optimal and resources readily available, selection may not be able to increase a female's fecundity as egg numbers may already be at maximum (Credland, 1968; Chapter 5). We also

investigated the effects of macronutrients on reproductive success in moths reared on diets varying in protein and carbohydrate (Chapter 6). Carbohydrate content was found to have significant effects on male and female life-history traits, as well as fitness. Protein is not often found in their natural diet, and the sexes may not be adapted to the amounts of protein supplied through the geometric framework. Previous research has proposed the lethal protein hypothesis (Simpson and Raubenheimer, 2007, 2009; Jensen *et al.*, 2015), suggesting that high intake of protein can cause reduction in lifespan. We found that lifespan in male and female *P. interpunctella* is favoured at a 1:2 ratio or 1:5 ratio of P:C respectively, providing further evidence to support the importance of dietary carbohydrates, and the negative consequences on lifespan of protein (Chapter 6).

7.6 Future Directions

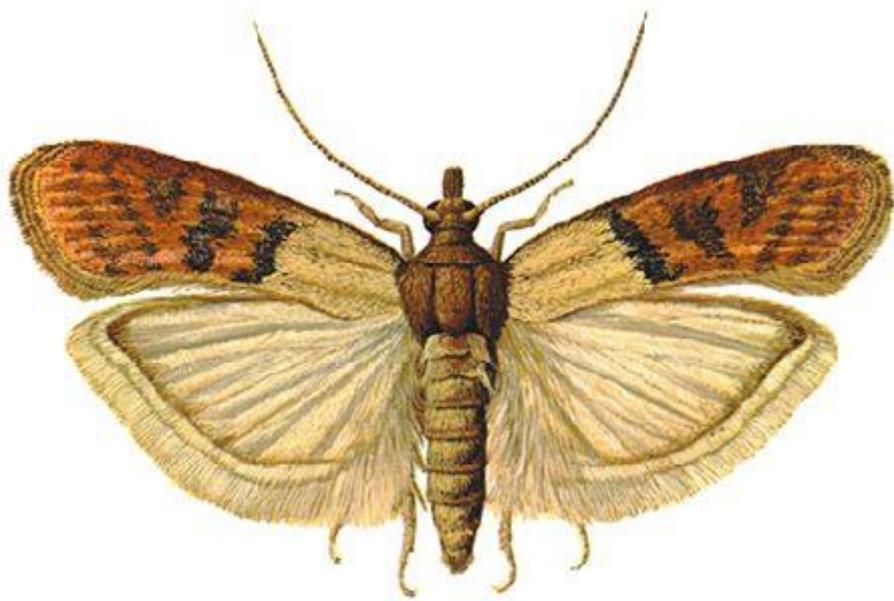
It is evident that pre-copulatory selection is of high importance to *P. interpunctella* males to ensure males gain successful matings (Chapter 2). Further work could examine sexual selection over mate choice and/ or attractiveness within the diverged FB and MB populations. Pheromones are released by *P. interpunctella* females to indicate receptivity to mating (Brady and Smithwick, 1968; Grant *et al.*, 1975). Behavioural studies utilising attract-and-kill chemo-attractants (Campos and Phillips, 2013) could be conducted, to determine if males from either treatment locate 'receptive females' quicker. As there is increased intrasexual competition for the limiting resource of females in MB populations, we may expect to see MB males locating traps (i.e receptive females) quicker than FB males.

Furthermore, a recent study examining sexual selection in the red flour beetle, *Tribolium castaneum* after evolving for many years under MB or FB regimes, as well as

enforced monogamy and polyandry highlights the importance of sexual selection (Lumley *et al.*, 2015). By subjecting the varying diverged lines to inbreeding using full sib matings, it was found that sexual selection maintained overall population fitness, as MB and polyandrous lines were 40% more resilient to extinction rates compared to FB and monogamous lines respectively. Likewise, a similar experiment could be conducted with our diverged MB and FB experimental evolution populations of *P. interpunctella*. By enforcing inbreeding and thereby exposing recessive deleterious alleles (Pusey and Wolf, 1996), we would make the prediction that higher levels of sexual selection faced by moths in the MB lines would increase the likelihood of survival. This would be due to sexual selection effectively reducing the mutational load through promotion and fixation of advantageous alleles in these populations (Lumley *et al.*, 2015).

7.7 Conclusions

In conclusion, this thesis has found that sexual conflict and sexual selection in *P. interpunctella* can lead to divergence in reproductive success, immune response and sperm competitive ability. Due to increased competition for matings, sexual selection also likely acts as a non-mutually exclusive force, leading to moths deriving from male-biased populations having higher reproductive success, and possibly also immune function. Dietary protein and carbohydrates are shown to be a further source of conflict for males and females, corroborating the divergence between the sexes over shared life-history traits and related fitness measures. Our research has highlighted that perhaps future studies should focus on the effects of both sexual conflict and sexual selection in tandem, but how to disentangle the effects of one from the other remains a challenge.



8. Appendices

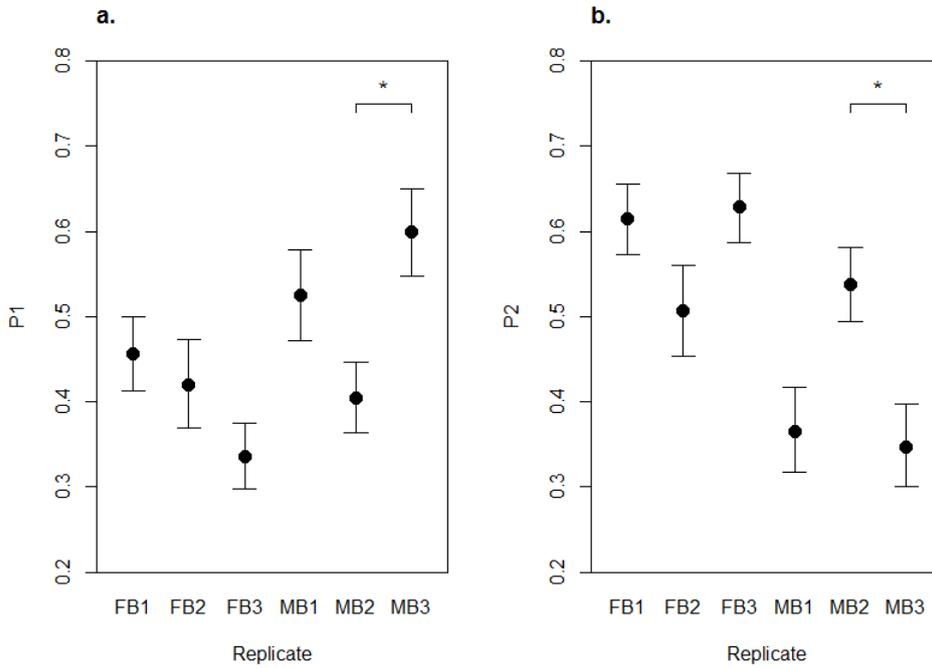


Figure 3.1A: Mean P_1 (a) and P_2 (b) in each of the replicates of female biased (FB), or male biased (MB) sex ratio treatments. *Significant differences between replicates. MB2 significantly differs from MB3 in both P_1 and P_2 mating roles. Error bars $\pm 1SE$.

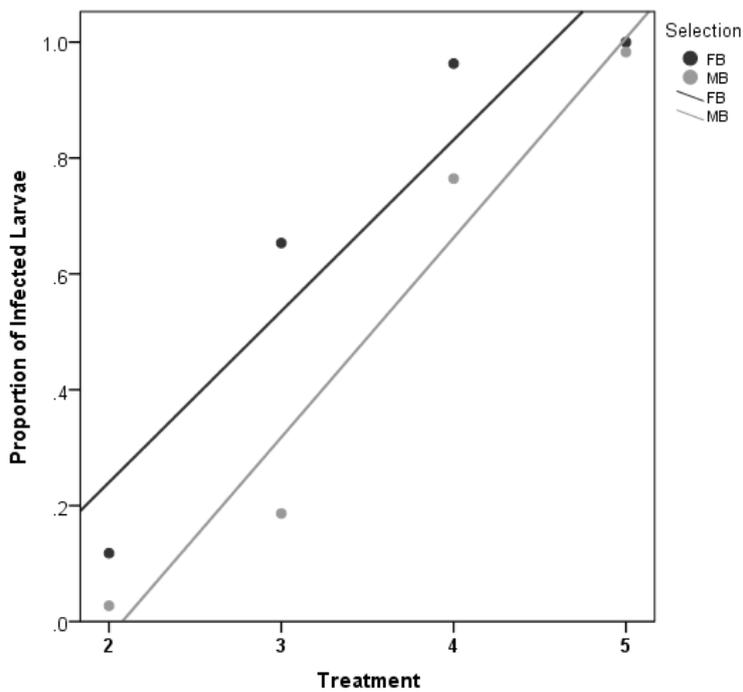


Figure 4.1A: Scatterplot of mean proportion of infected larvae in female biased (FB; black dots) and male biased populations (MB; grey dots) for each of the dose treatments. Treatments are: 2 = 0.0025%; 3 = 0.025%; 4 = 0.25% and 5 = 2.5% serial dilutions of *P. interpunctella* granulosis virus (*PiGV*).

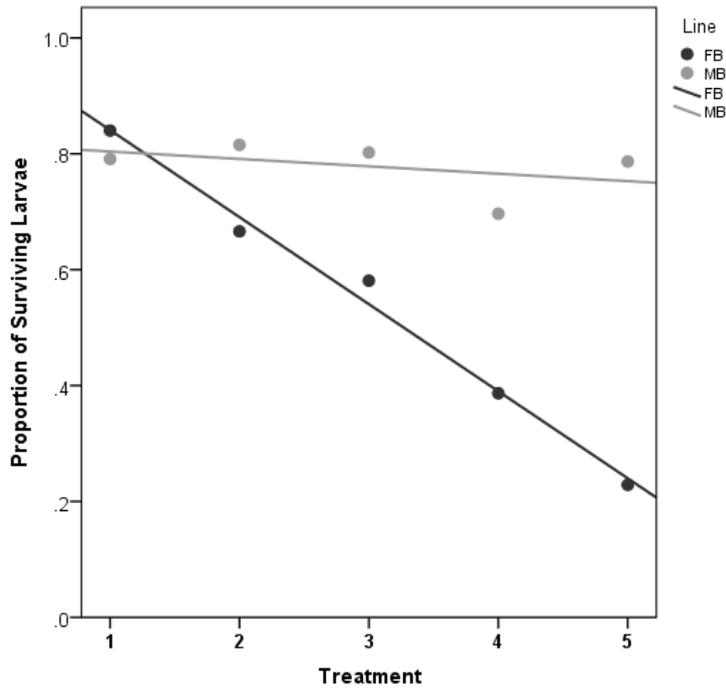


Figure 4.2A: Scatterplot of mean proportion of surviving larvae from female biased (FB; black dots) and male biased populations (MB; grey dots) for each viral dose. Dose treatments are: 1 = Control; 2 = 0.0025%; 3 = 0.025%; 4 = 0.25% and 5 = 2.5% serial dilution of *PiGV*.

Table 6.1A: Protein (P) and carbohydrate (C) composition of the 24 artificial diets used in our feeding experiment. The total nutrient in each diet is given as the sum of the percentage P and percentage C, with the remaining percentage consisting of indigestible crystalline cellulose.

Percentage Composition				
Protein (P)	Carbohydrate (C)	P+C	P:C ratio	Diet Number
10	2	12	05:01	1
30	6	36	05:01	2
50	10	60	05:01	3
70	14	84	05:01	4
9	3	12	03:01	5
27	9	36	03:01	6
45	15	30	03:01	7
63	21	84	03:01	8
6	6	12	01:01	9
18	18	36	01:01	10
30	30	60	01:01	11
42	42	84	01:01	12
3	9	12	01:03	13
9	27	36	01:03	14
15	45	60	01:03	15
21	63	84	01:03	16
2	10	12	01:05	17
6	30	36	01:05	18
10	50	60	01:05	19
14	70	84	01:05	20
1.33	10.66	12	01:08	21
4	32	36	01:08	22
6.66	53.33	60	01:08	23
9.33	74.66	84	1:8	24

References

- Adler, M.I., Cassidy, E.J., Fricke, C. and Bonduriansky, R. (2013) The lifespan-reproduction trade-off under dietary restriction is sex-specific and context-dependent. *Experimental Gerontology* **48**, p.539- 548.
- Ahmed, A.M., Baggott, S.L., Maingon, R. and Hurd, H. (2002) The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *OIKOS* **97**, p.371- 377.
- Andersson, M. (1982) Female choice selects for extreme tail length in a widowbird. *Nature* **299** (28), p.818- 820.
- Andersson, M.B. (1994). *Sexual selection*. Princeton University Press.
- Andersson, M. and Simmons, L.W. (2006) Sexual selection and mate choice. *Trends in Ecology and Evolution* **21** (6), p.296- 302.
- Archer, C.R., Zajitschek, F., Sakaluk, S.K., Royle, N.J. and Hunt, J. (2012) Sexual selection affects the evolution of lifespan and aging in the decorated cricket *Gryllodes sigillatus*. *Evolution* **66** (10), p.3088- 3100.
- Ardia, D.R., Gantz, J.E., Schneider, B.C. and Strebler, S. (2012) Costs of immunity in insects: an induced immune response increases metabolic rate and decreases microbial activity. *Functional Ecology* **26**, p732- 739.
- Arnqvist, G. and Nilsson, T. (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour* **60**, p.145- 164.
- Arnqvist, G. and Rowe, L. (1995) Sexual conflict and arms races between the sexes: a morphological adaption for control of mating in a female insect. *Proceedings of the Royal Society London B: Biological Sciences* **261**, p.123- 127.

- Arnqvist, G. and Rowe, L. (2002a) Correlated evolution of male and female morphologies in water striders. *Evolution* **56** (5), p.936- 947.
- Arnqvist, G. and Rowe, L. (2002b) Antagonistic coevolution between the sexes in a group of insects. *Nature* **415**, p.787- 789.
- Arnqvist, G. and Rowe, L. (2013) *Sexual conflict*. Princeton University Press.
- Arnqvist, G. and Tuda, M. (2010) Sexual conflict and the gender load: correlated evolution between population fitness and sexual dimorphism in seed beetles. *Proceedings of the Royal Society B: Biological Sciences* **277**, p.1345- 1352.
- Bachtrog, D. (2006) A dynamic view of sex chromosome evolution. *Current Opinion in Genetics and Development* **16**, p.578- 585.
- Bachtrog, D., Kirkpatrick, M., Mank, J.E., McDaniel, S.F., Pires, J.C., Rice, W.R. and Valenzuela, N. (2011) Are all sex chromosomes created equal? *Trends in Genetics* **27** (9), p.350- 357.
- Barnes, A.I. and Siva-Jothy, M.T. (2000). Density-dependant prophylaxis in the mealworm beetle *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): cuticular melanisation is an indicator of investment in immunity. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, p.177- 182.
- Bateman, A.J. (1948) Intra-sexual selection in *Drosophila*. *Heredity* **2**, p.349- 368.
- Bauerfeind, S.S. and Fischer, K. (2005) Effects of food stress and density in different life stages on reproduction in a butterfly. *OIKOS* **111** (3), p.514- 524.
- Berger, D., Walters, R. and Gotthard, K. (2008) What limits insect fecundity? Body size- and temperature-dependant egg maturation and oviposition in a butterfly. *Functional Ecology* **22**, p.523- 529.

- Berger, D., Olofsson, M., Gotthard, K., Wiklund, C. and Friberg, M. (2012) Ecological constraints on female fitness in a phytophagous insect. *The American Naturalist* **180** (4), p.464- 480.
- Berger, D., Berg, E.C., Widegren, W., Arnqvist, G. and Makalkov, A.A. (2014) Multivariate intralocus sexual conflict in seed beetles. *Evolution* **68** (12), p.3457- 3469.
- Bertram, S.M. and Rook, V. (2012) Relationship between condition, aggression, signalling, courtship and egg-laying in the field cricket, *Gryllus assimilis*. *Ethology* **118**, p.360- 372.
- Bernasconi, G. and Keller, L. (2001) Female polyandry affects their sons reproductive success in the red flour beetle *Tribolium castaneum*. *Journal of Evolutionary Biology* **14**, p.186- 193.
- Birkhead, T.R. (1998) Cryptic female choice: criteria for establishing female sperm choice. *Evolution* **52** (4), p.1212- 1218.
- Birkhead, T.R. and Møller, A.P. (1998) *Sperm competition and sexual selection*. London: Academic Press.
- Birkhead, T.R., Hosken, D.J. and Pitnick, S. (2009) *Sperm biology: an evolutionary perspective*. Academic Press.
- Bissoondath, C.J. and Wiklund, C. (1996) Effects of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Peris napi* and *Peris rapae* (Lepidoptera: Pieridae). *Functional Ecology* **10** (4), p.457- 464.
- Bjork, A., Starmer, W. T., Higginson, D. M., Rhodes, C. J. & Pitnick, S. (2007) Complex interactions with females and rival males limit the evolution of sperm offence and defence. *Proceedings of the Royal Society B: Biological Sciences* **274**, p.1779- 1788.

- Blackburn, G.S., Albert, A.Y.K. and Otto, S.P. (2010) The evolution of sex ratio adjustment in the presence of sexually antagonistic selection. *The American Naturalist* **176** (3), p.264- 275.
- Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology* **75** (4), p.385- 407.
- Boggs, C.L. (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* **23**, p.27- 37.
- Bonduriansky, R., Maklakov, A., Zajitschek, F. and Brooks, R. (2008) Sexual selection, sexual conflict and the evolution of ageing and lifespan. *Functional Ecology* **22**, p.443- 453.
- Bonduriansky, R. and Chenoweth, S.F. (2009) Intralocus sexual conflict. *Trends in Ecology and Evolution* **24** (5), p.280- 288.
- Boorman, E. and Parker, G.A. (1976) Sperm (ejaculate) competition in *Drosophila melanogaster* and the reproductive value of females to males in relation to female age and mating success. *Ecological Entomology* **1**, p.145- 155.
- Boots, M. and Begon, M. (1993). Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Functional Ecology* **7** (5), p528 -534.
- Boots, M. and Begon, M. (1994) Resource limitation and the lethal and sublethal effects of a viral pathogen in the Indian meal moth *Plodia interpunctella*. *Ecological Entomology* **19**, p319- 326.
- Boots, M. and Roberts, K.E. (2012) Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. *Proc. R. Soc. B.* **279**, p4009- 4014.
- Brady, E.U. and Smithwick, E.B. (1968) Production and release of sex attractant by the female Indian meal moth, *Plodia interpunctella*. *Annals of the Entomological Society of America* **61** (5), p.1260- 1265.

- Brown, W.D. (1997) Courtship feeding in tree crickets increases insemination and female reproductive lifespan. *Animal Behaviour* **54**, p.1369- 1382.
- Bunning, H., Rapkin, J., Bekker, L., Archer, C.R., Jensen, K. and Hunt, J. (2015) Protein and carbohydrate intake influence sperm number and fertility in male cockroaches, but not sperm viability. *Proceedings of the Royal Society B: Biological Sciences* **282**, p.1- 8.
- Byrne, P. G., Roberts, J. D. & Simmons, L. W. 2002. Sperm competition selects for increased testes mass in Australian frogs. *Journal of Evolutionary Biology* **15**, p.347- 355.
- Calsbeek, R. and Sinervo, B. (2004) Within-clutch variation in offspring sex determined by differences in sire body size: cryptic male choice in the wild. *Journal of Evolutionary Biology* **17**, p.464- 470.
- Campos, M. and Phillips, T.W. (2013) Laboratory evaluation of attract-and-kill formulations against the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). *Journal of Stored Products Research* **52**, p.12- 20.
- Chapman, T. (2001) Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity* **87**, p.511-521.
- Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L. (2003) Sexual conflict. *Trends in Ecology and Evolution* **18** (1), p.41- 47.
- Chapman, T., Miyatake, T., Smith, H.K. and Partridge, L. (1998) Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. *Proceedings of the Royal Society of London B: Biological Sciences* **265**, p.1879- 1894.
- Chapman, T, and Davies, S.J. (2004) Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides* **25**, p.1477- 1490.

- Charlesworth, D. and Charlesworth, B. (1980) Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes. *Genetical Research* **35** (2), p.205- 214.
- Chippindale, A.K., Alipaz, J.A., Chen, H.W and Rose, M.R. (1997) Experimental evolution of accelerated development in *Drosophila* 1. Developmental speed and larval survival. *Evolution* **51** (5), p.1536- 1551.
- Chippindale, A.K., Gibson, J.R. and Rice, W. (2001) Negative genetic correlation for adult fitness between the sexes reveals ontogenetic conflict in *Drosophila*. *Proceedings of the National Academy of Science of the United States of America* **98**, p.1671- 1675.
- Clark, A. G., Aguadé, M., Prout, T., Harshmad, L. G. & Langley, C.H. (2005) Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. *Genetics* **139**, p.189- 201.
- Clark, R.M., McConnell, A., Zera, A.J. and Behmer, S.T. (2013) Nutrient regulation strategies differ between cricket morphs that trade-off dispersal and reproduction. *Functional Ecology* **27**, p. 1126- 1133.
- Clark, R.M., Zera, A.J. and Behmer, S.T. (2015) Nutritional physiology of life-history trade-offs: how food protein-carbohydrate content influences life-history traits in the wing-polymorphic cricket *Gryllus firmus*. *Journal of Experimental Biology* **218**, p. 298- 308.
- Clutton-Brock, T.H., Albon, S.D., Gibson, R.M. and Guinness, F.E. (1979) The logical stag: adaptive aspects of fighting in red deer (*Cervus elaphus* L.). *Animal Behaviour* **27**, p.211- 225.
- Cook, P.A. (1996) Sperm competition in butterflies and moth (Lepidoptera). Ph.D. thesis. University of Liverpool.

- Cook, P.A. (1999) Sperm numbers and female fertility in the moth *Plodia interpunctella* (Hübner) (Lepidoptera; Pyralidae). *Behavioural Ecology and Sociobiology* **36**, p.261- 268.
- Cook, P.A. and Gage, M.J.G. (1995) Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera, Pyralidae). *Behavioural Ecology and Sociobiology* **36**, p.261- 268.
- Cook, P.A. and Wedell, N. (1996) Ejaculate dynamics in butterflies: a strategy for maximising fertilisation success? *Proceedings of the Royal Society London B: Biological Sciences* **263**, p.1047- 1051.
- Cook, P.A., Harvey, I.F. and Parker, G.A. (1997) Predicting variation in sperm precedence. *Philosophical Transactions of the Royal Society London B: Biological Sciences* **352**, p.771- 780.
- Cook, P. A. & Wedell, N. (1999) Non-fertile sperm delay female re-mating. *Nature* **397**, p.496.
- Cotter, S.C., Raubenheimer, D. and Wilson, K. (2011) Macronutrient balance mediates trade-offs between immune function and life-history traits. *Functional Ecology* **25**, p.186- 198.
- Cotton, S., Small, J., Hashim, R. and Pomiankowski, A. (2010) Eyespan reflects reproductive quality in wild stalk-eyed flies. *Evolutionary Ecology* **24**, p.83- 95.
- Cox, R.M., Skelly, S.L. and John-Alder, H.B. (2003) A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution* **57** (7), p.1653- 1669.

- Cox, R.M. and Calsbeek, R. (2009) Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *The American Naturalist* **173** (2), p.176- 187.
- Cox, R.M. and Calsbeek, R. (2010) Severe costs of reproduction persist in Anolis lizards despite the evolution of a single-egg clutch. *Evolution* **64** (5), p.193- 197.
- Credland, P.F. (1986) Effect of host availability on reproductive performance in *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Product Research* **22** (1), p.49- 54.
- Darwin, C. (1859) *On the origin of species by means of natural selection*. London: John Murray.
- Darwin, C. (1871) *The descent of man and selection in relation to sex*. (2nd Ed) John Murray. London.
- Daly, M. (1978) The cost of mating. *The American Naturalist* **112**, p.771- 774.
- Demary, K. C. & Lewis, S. M. (2007) Male courtship attractiveness and paternity success in *Photinus Greeni* fireflies. *Evolution* **61**, p.431- 439.
- Dewsbury, D. (1982) Ejaculate cost and mate choice. *American Naturalist* **119**, p.601- 610.
- Drummond, B. (1984) Multiple mating and sperm competition in the Lepidoptera. In: *Sperm competition and the evolution of animal mating systems* (Ed. by Smith, R.L.), p.291- 370.
- Duan, L. and Otvos, I.S. (2001) Influence of larval age and virus concentration on mortality and sublethal effects of a nucleopolyhedrovirus on the western spruce budworm (Lepidoptera: Tortricidae). *Environmental Entomology* **30** (1), p136- 146.
- Dugdale, J.S. (1974) Female genital configuration in the classification of Lepidoptera. *New Zealand Journal of Zoology* **1**, p.127- 146.

- Eberhard, W.G. (1996) *Female control: sexual selection by cryptic female choice*.
Princeton University Press.
- Edgar, B.A. (2006) How flies get their size: genetics meets physiology. *Nature Reviews Genetics* **7**, p.907- 916.
- Einum, S. and Fleming, I.A. (2000) Highly fecund mothers sacrifice offspring survival to maximise fitness. *Nature* **405**, p.565- 567.
- Elias, D.O., Sivalinghem, S., Mason, A.C., Androde, M.C.B., and Kasumovic, M.M. (2014) Mate-guarding courtship behaviour: tactics in a changing world. *Animal Behaviour* **97**, p.25- 33.
- Elgar, M.A. and Fahey, B.F. (1996) Sexual cannibalism, competition and size dimorphism in the orb-weaving spider *Nephila plumipes* Latreille (Araneae: Araneoidea). *Behavioural Ecology* **7** (2), p.195- 198.
- Ellegren, H. and Parsch, J. (2007) The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics* **8** (9), p.689- 698.
- Ellegren, H. (2011) Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. *Nature Reviews Genetics* **12**, p.157- 166.
- Emlen, D.J., Marangelo, J., Ball, B. and Cunningham, C.W. (2005) Diversity in the weapons of sexual selection: horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). *Evolution* **59** (5), p.1060- 1084.
- Emlen, D.J., Szafran, Q., Corley, L.S. and Dworkin, I. (2006) Insulin signalling and limb patterning: candidate pathways for the origin and evolutionary diversification of beetlehorns. *Heredity* **97**, p.179- 191.
- Emlen, D.J. (2008) The evolution of animal weapons. *Annual Review of Ecology, Evolution and Systematics* **39**, p.387- 413.

- Emlen, S.T. and Oring, L.W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, p.215- 233.
- Evans, J. P., Zane, L., Francescato, S. & Pilastro, A. (2003) Directional postcopulatory sexual selection revealed by artificial insemination. *Nature* **421**, p.360- 363.
- Fairbairn, D.J. (1997) Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics* **28**, p.659- 687.
- Fanson, B.G., Weldon, C.W., Pérez-Staples, D., Simpson, S.J. and Taylor, P.W. (2009) Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell* **8**, p.415- 523.
- Fanson, B.G. and Taylor, P.W. (2012) Protein:carbohydrate ratios explain lifespan patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. *Age* **34**, p.1361- 1368.
- Fedina, T.Y. and Lewis, S.M. (2006) Proximal traits and mechanisms for biasing paternity in the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Behavioural Ecology and Sociobiology* **60**, p.844- 853.
- Fedorka, K.M. and Mousseau, T.A. (2004) Female mating bias results in conflicting sex-specific offspring fitness. *Nature* **429**, p.65- 67.
- Fellous, S. and Lazzaro, B.P. (2010) Larval food quality affects adult (but not larval) immune gene expression independent of effects on general condition. *Molecular ecology* **19**, p.1462- 1468.
- Ferdig, M.T., Beerntsen, B.T., Spray, F.J., Li, J. and Christensen, B.M. (1993) Reproductive costs associated with resistance in a mosquito filarial worm system. *American Journal of Tropical Medicine Hygiene* **49**, p.756- 762.

- Firman, R.C. and Simmons, L.W. (2008) Polyandry facilitates post copulatory inbreeding avoidance in house mice. *Evolution* **62** (3), p.603- 611.
- Fisher, R.A. (1915) The evolution of sexual preference. *Eugenics Review* **7**, p.184- 192.
- Fisher, R.A. (1930) *The genetical theory of natural selection*. Oxford: Clarendon Press.
- Flatt, T. (2011) Survival costs of reproduction in *Drosophila*. *Experimental Gerontology* **46**, p.369- 375.
- Foerster, K., Coulson, T., Sheldon, B.C., Pemberton, J.M., Clutton-Brock, T.H. and Kruuk, L.E.B. (2007) Sexually antagonistic genetic variation for fitness in red deer. *Nature* **447**, p.1107- 1111.
- Friberg, U., Lew, T. A., Byrne, P. G. & Rice, W. R. (2005) Assessing the potential for an ongoing arms race within and between the sexes: Selection and heritable variation. *Evolution* **59**, p.1540- 1551.
- Fricke, C., Martin, O.Y., Bretman, A., Bussière, L.F. and Chapman, T. (2010) Sperm competitive ability and indices of lifetime reproductive success. *Evolution* **64** (9), p.2746- 2757.
- Frid, L. and Myers, J.H. (2002) Thermal ecology of western tent caterpillars *Malacosoma californicum pluviale* and infection by nucleopolyhedrovirus. *Ecological Entomology* **27**, p.665- 673.
- Friedländer, M. (1997) Control of the eupyrene- apyrene sperm dimorphism in Lepidoptera. *Journal of Insect Physiology* **43** (12), p.1085- 1092.
- Friedländer, M. and Wahrman, J. (1970) The spindle as a basal body distributor. A study in the meiosis of the male silkworm moth, *Bombyx mori*. *Journal of Cell Science* **7**, p.65- 89.

- Fritzsche, K. and Arnqvist, G. (2015) The effects of male phenotypic condition on reproductive output in a sex role-reversed beetle. *Animal Behaviour* **102**, p.209- 215.
- Fromhage, L. and Schneider, J.M. (2005) Safer sex with feeding females: sexual conflict in a cannibalistic spider. *Behavioural Ecology* **16** (2), p.377- 382.
- Fuller, R.C., Houle, D. and Travis, J. (2005) Sensory bias as an explanation for the evolution of mate preferences. *The American Naturalist* **166** (4), p.437- 446.
- Gage, M.J.G. (1995) Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proceedings of the Royal Society London B: Biological Sciences* **261**, p.25- 30.
- Gage, M.J.G. and Cook, P.A. (1994) Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidea). *Functional Ecology* **8** (5), p.594- 599.
- Gall, M.L. and Behmer, S.T. (2014) Effects of protein and carbohydrate on an insect herbivore: the vista from a fitness landscape. *Integrative and Comparative Biology* **54** (5), p.942- 954.
- Gallach, M. and Betrán, E. (2011) Intralocus sexual conflict resolved through gene duplication. *Trends in Ecology and Evolution* **26** (5), p.222- 228.
- García-Barros, E. (2000) Body size, egg size and their interspecific relationships with ecological and life-history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biological Journal of the Linnean Society* **70**, p.251- 284.
- Gasparini, C. and Pilastro, A. (2011) Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proceedings of the Royal Society B: Biological Sciences* **278**, p.2495- 2501.

- Gavrilets, S. (2014) Is sexual conflict an “engine of speciation”? *Cold Spring Harbor Perspective in Biology* **6**, a017723.
- Gershman, S.N., Hunt, J. and Sakaluk, S.K. (2013) Food fight: sexual conflict over free amino acids in the nuptial gifts of male decorated crickets. *Journal of Evolutionary Biology* **26**, p.693- 704.
- Grant, G.G. and Brady, U.E. (1975) Courtship behaviour of phycitid moths. I. Comparison of *Plodia interpunctella* and *Cadra cautella* and role of male scent glands. *Canadian Journal of Zoology* **53**, p.813- 826.
- Grant, G.G., Smithwick, E.B. and Brady, U.E. (1975) Courtship behaviour of phycitid moths. II. Behavioural and pheromonal isolation of *Plodia interpunctella* and *Cadra cautella* in the laboratory. *Canadian Journal of Zoology* **53**, p.827- 832.
- Gwynne, D.T. (2001) *Katydid and bushcrickets: reproductive behaviour and evolution of the Tettigoniidae*. Cornell University Press.
- Gwynne, D.T. (2008) Sexual conflict over nuptial gifts in insects. *Annual Review of Entomology* **53**, p.83- 101.
- Hall, M.D., Bussi re, L.F. and Brooks, R. (2008) The effect of diet quality and wing morph on male and female reproductive investment in a nuptial feeding ground cricket. *PLoS ONE* **3** (10), e3437
- Hamilton, W.D. and Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science* **218**, p.384- 387.
- Harano, T., Nakamoto, Y. & Miyatake, T. (2008) Sperm precedence in *Callosobruchus chinensis* estimated using the sterile male technique. *Japan Ethological Society* **26**, p.201- 206.

- Harano, T., Okada, K., Nakayama, S., Miyatake, T. and Hosken, D.J. (2010) Intralocus sexual conflict unresolved by sex-limited trait expression. *Current Biology* **20**, p.2036- 2039.
- Harrison, S.J., Thomson, I.R., Grant, C.M. and Bertram, S.M. (2013) Calling, courtship, and condition in the fall field cricket, *Gryllus pennsylvanicus*. *PLoS ONE* **8** (3), e60356.
- Hau, M. (2007) Regulation of male traits by testosterone: implications for the evolution of vertebrate life-histories. *BioEssays* **29** (2), p.133- 144.
- Hayward, A. and Gillooly, J.F. (2011) The cost of sex: quantifying energetic investment in gamete production by males and females. *PLoS One* **6** (1), e16557.
- Honěk, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *OIKOS* **66** (3), p.483- 492.
- Hosken, D. J. (1997) Sperm competition and the evolution of testis and sperm size in bats. *Proceedings of the Royal Society B: Biological Sciences* **264**, p.385- 392.
- Hosken, D.J. (2011) Gene duplication might not resolve intralocus sexual conflict. *Trends in Ecology and Evolution* **26** (11), p.556- 557.
- Hosken, D.J. and Ward, P.I. (2001) Experimental evidence for testis size evolution via sperm competition. *Ecology Letters* **4**, p.10- 13.
- Hosken, D.J., Garner, T.W.J., Tregenza, T., Wedell, N. and Ward, P.I. (2003) Superior sperm competitors sire higher-quality young. *Proceedings of the Royal Society of London B: Biological Sciences* **270**, p.1933- 1938.
- Hosken, D.J. and Stockley, P. (2005) Sexual conflict. *Current Biology* **15** (14), p535- 536.
- Hosken, D. J., Taylor, M. L., Hoyle, K., Higgins, S. & Wedell, N. (2008) Attractive males have greater success in sperm competition. *Current Biology* **18**, p.553- 554.

- Houslay, T.M., Hunt, J., Tinsley, M. and Bussière, L.F. (2015) Sex differences in the effects of juvenile and adult diet on age-dependant reproductive effort. *Journal of Evolutionary Biology* **28** (5), p.1067- 1079.
- House, C. M. & Simmons, L. W. (2006) Offensive and defensive sperm competition roles in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Behavioural Ecology and Sociobiology* **60**, p.131- 136.
- Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L and Bussière, L.F. (2004) High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**, p.1024- 1027.
- Ingleby, F.C., Lewis, Z. and Wedell, N. (2010) Level of sperm competition promotes evolution of male ejaculate allocation patterns in a moth. *Animal Behaviour* **80**, p.37- 43.
- Jaramillo, S.L., Mehlferber, E. and Moore, P.J. (2015) Life-history trade-offs under different larval diets in *Drosophila suzukii* (Diptera: Drosophilidae). *Physiological Entomology* **40**, p.2- 9.
- Jennions, M.D. and Petrie, M. (2000) Why do females mate multiply? A review of the genetic benefits. *Biological reviews* **75**, p.21- 6.4
- Jensen, K., McClure, C., Priest, N.K. and Hunt, J. (2015) Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in *Drosophila melanogaster*. *Aging Cell*, p.1- 11.
- Jiménez-Cortés, J.G., Serrano-Meneses, M.A., Córdoba-Aguilar, A. (2012) The effects of food shortage during larval development on adult body size, body mass, physiology and development time in a topical damselfly. *Journal of Insect Physiology* **58**, p.318- 326.

- Jones, A.G. Rosenqvist, G., Bergland, A. and Avise, J.C. (2005) The measurement of sexual selection using Bateman's principles: an experimental test in the sex-role reversed pipefish *Syngnathus typhle*. *Integrative Comparative Biology* **45**, p.874- 884.
- Jones, A.G. (2009) On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution* **63** (7), p.1673- 1684.
- Jönsson, K.I. (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *OIKOS*, p.57- 66.
- Karino, K., Shimada, Y., Kudo, H. and Sato, A. (2002) Relative importance of the area and intensity of the orange spots of male guppies *Poecilia reticulata* as mating traits preferred by females. *Journal of Fish Biology* **77**, p.299- 307.
- Karl, I., Stoks, R., de Block, M., Janowitz, S.A. and Fischer, K. (2011) Temperature extremes and butterfly fitness: conflicting evidence from life-history and immune function. *Global Exchange Biology* **17**, p.676- 687.
- Katsuki, M., Harano, T., Miyatake, T., Okado, K. and Hosken, D.J. (2012) Intralocus sexual conflict and offspring sex ratio. *Ecology Letters* **15**, p.193- 197.
- Keller, L. & Reeve, H. K. (1995) *Why do females mate with multiple males? The sexually selected sperm hypothesis*. *Advances in the Study of Behavior* **24**, p.291- 315.
- Kelly, C.D. (2004) Allometry and sexual selection of male weaponry in wellington tree weta, *Hemideina crassidens*. *Behavioural Ecology* **16** (1), p.145- 152.
- Kerje, S., Carlborg, Ö., Jacobsson, L., Schütz, K., Hartmann, C., Jensen, P. and Andersson, L. (2003) The twofold difference in adult size between the Red Junglefowl and White Leghorn chickens is largely explained by a number of QTLs. *Animal Genetics* **34**, p.264- 274.

- Khazaeli, A.A. and Curtsinger, J.W. (2013) Pleiotropy and life-history evolution in *Drosophila melanogaster*: uncoupling lifespan and early fecundity. *Journal of Gerontology: Biological Sciences* **68** (5), p.546- 553.
- Kirkpatrick, M. (1982) Sexual selection and the evolution of female choice. *Evolution* **36** (1), p.1- 12.
- Kokko, H. and Jennions, M.D. (2008) Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* **21**, p.919- 948.
- Kraus, C., Pavard, S. and Promislow, D.E.L. (2013) The sex-lifespan trade-off decomposed: why large dogs die young. *The American Naturalist* **181** (4), p.492- 505.
- Kvarnemo, C. and Ahnesjö, I. (1996) The dynamics of operational sex ratios and competition for mates. *Trends in Ecology and Evolution* **11** (10), p.404- 408.
- Lande, R. (1980) Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution* **34** (2), p.292- 305.
- Lande, R. and Arnold, S.J. (1983) The measurement of selection on correlated characters. *Evolution* **37**, p.1210- 1226.
- Lawniczak, M.K.N., Barnes, A.I., Linklater, J.R., Boone, J.M., Wigby, S. and Chapman, T. (2007). Mating and immunity in invertebrates. *Trends in Ecology and Evolution* **22** (1), p.48- 55.
- Lazzaro, B.P. and Little, T.J. (2009) Immunity in a variable world. *Phil. Trans. R. Soc. B.* **364**, p15- 26.
- Leftwich, P.T., Edward, D.A., Alphey, L., Gage, M.J.G. and Chapman, T. (2012) Variation in adult sex ratio alters the association between courtship, mating frequency and paternity in the lek-forming fruitfly *Ceratitis capitata*. *Journal of Evolutionary Biology* **25**, p 1732- 1740.

- Lee, K.P. (2010) Sex-specific differences in nutrient regulations in a capital breeding caterpillar, *Spodoptera litura* (Fabricius). *Journal of Insect Physiology* **56**, p.1685- 1695.
- Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N. and Raubenheimer, D. (2008) Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proceedings of the National Academy of Science USA* **105**, p.2498- 2503.
- Lee, M.S., Albajes, R. and Eizaguirre, M. (2014) Mating behaviour of female *Tutu absoluta* (Lepidoptera; Gelechiidae): polyandry increases reproductive output. *Journal of Pest Science* **87**, p.429- 439.
- Lewis, S. M. & Austad, S. N. (1994) Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness. *Behavioural Ecology*, **5**, p.219- 224.
- Lewis, Z. (2005) Sexual selection and sexual conflict in the Lepitdoptera. Ph.D. thesis, University of Leeds.
- Lewis, Z., Wedell, N. and Hunt, J. (2011). Evidence for strong intralocus sexual conflict in the Indian meal moth, *Plodia interpunctella*. *Evolution* **65** (7), p.2085- 2097.
- Lewis, Z., Sasaki, H. and Miyatake, T. (2011b) Sex starved: do resource-limited males ensure fertilisation success at the expense of pre-copulatory mating success? *Animal Behaviour* **81**, p.579- 583.
- Lewis, S. and South, A. (2012) The evolution of animal nuptial gifts. *Advances in the Study of Behaviour* **44**, p.53- 97.
- Lewis, Z., Lizé, A. and Wedell, N. (2013) The interplay between different stages of reproduction in males of the moth *Plodia interpunctella*. *Animal Behaviour* **86**, p.917- 922.

- Long, T.A.F., Montgomerie, R. and Chippindale, A.K. (2006) Quantifying the gender load: can population crosses reveal interlocus sexual conflict. *Philosophical Transactions of the Royal Society B: Biological Sciences* **361**, p.363- 374.
- Loyau, A., Jalme, M.S., Cagniant, C. and Sorci, G. (2005) Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). *Behavioural Ecology and Sociobiology* **58**, p.552- 557.
- Mank, J.E., Hosken, D.J. and Wedell, N. (2011) Some inconvenient truths about sex chromosome dosage compensation and the potential role of sexual conflict. *Evolution* **65** (8), p.2133- 2144.
- McNamara, K.B., van Lieshout, E., Jones, T.M and Simmons, L.W. (2013a) Age-dependant trade-offs between immunity and male, but not female, reproduction. *Journal of Animal Ecology* **82**, p.235- 244.
- Maklakov, A.A., Bilde, T. and Lubin, Y. (2004) Sexual selection for increased male body size and protandry in a spider. *Animal Behaviour* **68**, p.1041- 1048.
- Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessman, J., Clissold, F., Raubenheimer, D., Bonduriansky, R. and Brooks, R.C. (2008) Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Current Biology* **18**, p.1062- 1066.
- Martin, O.Y. and Hosken, D.J. (2003) The evolution of reproductive isolation through sexual conflict. *Nature* **423**, p.979- 982.
- Martin, L.B., Weil, Z.M. and Nelson, R.J. (2008). Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Phil. Trans. R. Soc. B.* **363**, p 321- 339.
- Matthews, I.M. and Magurran, A.E. (2000) Evidence for sperm transfer during sneaky mating in wild Trinidadian guppies. *Journal of Fish Biology* **56**, p.1381- 1386.

- May, C.M., Doroszuk, A. and Zwaan, B.J. (2015) The effect of developmental nutrition on lifespan and fecundity depends on the adult reproductive environment in *Drosophila melanogaster*. *Ecology and Evolution* **5** (6), p.1156- 1168.
- McCullough, E.L. and Emlen, D.J. (2013) Evaluating the costs of a sexually selected weapon: big horns at a small price. *Animal Behaviour* **86**, p.977- 985.
- McKean, K.A. and Nunney, L. (2001) Increased sexual activity reduces male immune function in *Drosophila melanogaster*. *PNAS* **98** (14), p7904- 7909.
- McNamara, K.B., van Lieshout, E., Jones, T.M and Simmons, L.W. (2013a) Age-dependant trade-offs between immunity and male, but not female, reproduction. *Journal of Animal Ecology* **82**, p 235- 244.
- McNamara, K.B., Wedell, N. and Simmons, L.W. (2013b) Experimental evolution reveals trade-offs between mating and immunity. *Biology Letters* **9** (4).
- Mealor, M.A. and Boots, M. (2007) An indirect approach to imply trade-off shapes: population level patterns in resistance suggest a decreasingly costly resistance mechanism in a model insect system. *Journal of Evolutionary Biology* **19**, p326- 330.
- Metaxakis, A. and Partridge, L. (2013) Dietary restriction extends lifespan in wild-derived populations of *Drosophila melanogaster*. *PLoS ONE* **8** (9), e74681.
- Moghadam, H.K., Pointer, M.A., Wright, A.E., Berlin, S. and Mank, J.E. (2012) W chromosome expression responds to female-specific selection. *Proceedings of the National Academy of the Sciences* **109** (21), p.8207- 8211.
- Mohandass, S., Arthur, F.H., Zhu, K.Y., and Throne, J.E. (2007). Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. *Journal of Stored Product Research* **43**, p302- 311
- Møller, A. P. (1988) Testes size, ejaculate quality and sperm competition in birds. *Biological Journal of the Linnean Society* **33**, p.273- 283.
- Möller, A.P. and Alatalo, R.V. (1999) Good genes effects in sexual selection. *Proceedings of the Royal Society London B: Biological Sciences*. **266**, p.85- 91.

- Møller, A.P., Christe, P. and Lux, E. (1999) Parasitism, host immune function and sexual selection. *The Quarterly Review of Biology* **74** (1), p.3- 20.
- Möller, A.P. and Jennions, M.D. (2001) How important are direct fitness benefits of sexual selection? *Naturwissenschaften* **88**, p.401- 415.
- Moret, Y. and Schmid-Hempel, P. (2000) Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**, p.1166- 1167.
- Morrow, E.H. and Gage, M.J.G. (2000) The evolution of sperm length in moths. *Proceedings of the Royal Society London B: Biological Sciences* **267**, p.307- 313.
- Nabi, M. N. & Harrison, R. A. (1984a) Laboratory tests on mating competition between thiotepa-sterilised and normal males of the potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Annals of Applied Biology* **104**, p.423- 426.
- Nabi, M. N. & Harrison, R. A. (1984b) Sterilisation of the potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) by fumigation with thiotepa. *Annals of Applied Biology* **104**, p.413- 421.
- Nakagawa, S., Lagisz, M., Hetor, K.L. and Spencer, H.G. (2012) Comparative and meta-analytic insights into life extension via dietary restriction. *Aging Cell* **11** (3), p.401- 409.
- Neems, R.M., Lazarus, J. and McLachlan, J. (1998) Lifetime reproductive success in a swarming midge: trade-offs and stabilising selection for male body size. *Behavioural Ecology* **9** (3), p.279- 286.
- Nestor, K.E., Noble, D.O., Zhu, J. and Moritsu, Y. (1996) Direct and correlated responses to long-term selection for increased body weight and egg production in Turkeys. *Poultry Science* **75**, p.1180- 1191.

- Nunney, L. (1996) The response to selection for fast larval development in *Drosophila melanogaster* and its effects on adult weight: an example of a fitness trade-off. *Evolution* **50** (3), p.1193- 1204.
- Nylin, S. and Gotthard, K. (1998) Plasticity in life-history traits. *Annual Review of Entomology* **43**, p.483- 492.
- Ortiz-Jiménez, I. and Cueva del Castillo, R. (2015) Nuptial gifts and female fecundity in the neotropical katydid *Conocephalus ictus* (Orthoptera; Tettigonidae). *Insect Science* **22**, p.106- 110.
- Parker, G.A. (1970a) The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. IV. Epigamic recognition and competition between males for the possession of females. *Behaviour* **37**, p.113- 139.
- Parker, G.A. (1970b) Sperm competition and its evolutionary consequences in the insects. *Biological Reviews* **45** (4), p.525- 567.
- Parker, G.A. (1979) Sexual selection and sexual conflict. In: *Selection and reproductive competition in insects* (Ed. by Blum, M.S. and Blum, N.A.). New York: Academic Press.
- Parker, G.A. (1982) Why are there so many tiny sperm – sperm competition and the maintenance of two sexes. *Journal of Theoretical Biology* **96**, p.281- 294.
- Parker G.A. (1984) *Sperm competition and the evolution of animal mating system*. London: Academic Press.
- Parker, G.A. (1990) Sperm competition games: raffles and roles. *Proceedings of the Royal Society London B: Biological Sciences* **242**, p.120- 126.
- Parker, G.A. (1998) Sperm competition and the evolution of ejaculates: towards a theory base. In *Sperm competition and sexual selection*. London Academic Press p.3- 54.

- Parker, G.A. (2006). Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society*. **361**, p.235- 259.
- Parker, G.A., Ball, M.A., Stockley, P. and Gage, M.J.G. (1996) Sperm competition games: individual assessment of sperm competition intensity by group spawners. *Proceedings of the Royal Society of London B: Biological Sciences* **263** (1375), p.1291- 1297.
- Parker, G.A., Ball, M.A., Stockley, P. and Gage, M.J.G. (1997) Sperm competition games: a prospective analysis of risk assessment. *Proceedings of the Royal Society of London B: Biological Sciences* **264** (1389), p.1793- 1802.
- Partidge, L., Prowse, N. and Pignatelli, P. (1999) Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Proceedings of the Royal Society London B: Biological Sciences* **266**, p.255- 261.
- Partridge, L., Gems, D. and Withers, D.J. (2005) Sex and death: what is the connection? *Cell* **120**, p.461- 472.
- Pekkala, N., Kotiaho, J.S. and Puurtinen, M. (2011) Laboratory relationships between adult lifetime reproductive success and fitness surrogates in a *Drosophila littoralis* population. *PLoS ONE* **6** (9), e24560
- Pennell, T.M. and Morrow, E.H. (2013) Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecology and Evolution* **3** (6), p.1819- 1834.
- Peretti, A.V. and Eberhard, W.G. (2010) Cryptic female choice via sperm dumping favours male copulatory courtship in a spider. *Journal of Evolutionary Biology* **23**, p.271- 281.
- Perry, J.C. and Rowe, L. (2012) Sexual conflict and antagonistic coevolution across water strider populations. *Evolution* **66** (2), p.544- 557.

- Perry, J.C., Sirot, L. and Wigby, S. (2013) The seminal symphony: how to compose an ejaculate. *Trends in Ecology and Evolution* **28** (7), p.414- 422.
- Pijpe, J., Fischer, K., Brakefield, P.M. and Zwaan, B.J. (2006) Consequences of artificial selection on pre-adult development for adult lifespan under benign conditions in the butterfly *Bicyclus anynana*. *Mechanisms of Ageing and Development* **127**, p.802- 807.
- Pilastro, A. and Bisazza, A. (1999) Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society London B: Biological Sciences*. **266**, p.1887- 1891.
- Pilastro, A., Simonato, M., Bisazza, A. and Evans, J.P. (2004) Cryptic female preference for colourful males in guppies. *Evolution* **58** (3), p.665- 669.
- Pincheira-Donoso, D. and Tregenza, T. (2011) Fecundity selection and the evolution of reproductive output and sex-specific body size in the *Liolaemus* lizard adaptive radiation. *Evolutionary Biology* **38**, p.197- 207.
- Pincheira-Donoso, D. and Hunt, J. (2015) Fecundity selection theory: concepts and evidence. *BioRxiv*, 015586.
- Pischedda, A. and Chippindale, A.K. (2006) Intralocus conflict diminishes the benefits of sexual selection. *PLoS Biology* **4** (11), e356.
- Pitnick, S. and Brown, W.D. (2000) Criteria for demonstrating female sperm choice. *Evolution* **54** (3), p.1052- 1056.
- Pitnick, S., Miller, G. T., Reagan, J. & Holland, B. (2001) Males' evolutionary responses to experimental removal of sexual selection. *Proceedings of the Royal Society B: Biological Sciences* **268**, p.1071- 1080.
- Pizzari, T. and Birkhead, T.R. (2000) Female feral fowl eject sperm of subdominant males. *Nature* **405**, p.787- 789.

- Pliske, T. (1975) Courtship behaviour of the monarch butterfly *Danaus plexippus* L. *Annals of the Entomological Society of America* **69**, p.143- 151.
- Popham, H.J.R., Shelby, K.S., Brandt, S.L. and Coudron, T.A. (2004) Potent virucidal activity in larval *Heliothis virescens* plasma against *Helicoverpa zea* single capsid nucleopolyhedrovirus. *Journal of General Virology* **85**, p2255- 2261.
- Preziosi, R.F., Fairbairn, D.J., Roff, D.A. and Brennan, J.M. (1996) Body size and fecundity in the waterstrider *Aquaris remigis*: a test of Darwin's fecundity advantage hypothesis. *Oecologia* **108**, p.424 – 432.
- Price, T.A.R. and Hosken, D.J. (2007) Evolution: good males are bad females. *Current Biology* **17** (5), p.168- 170.
- Price, T.A.R., Hodgson, D.J., Lewis, Z., Hurst, G.D.D. and Wedell, N. (2008) Selfish genetic elements promote polyandry in a fly. *Science* **322** (5905), p.1241- 1243.
- Pusey, A. and Wolf, M. (1996) Inbreeding avoidance in animals. *Trends in Ecology and Evolution* **11** (5), p.201- 206.
- Rajyaguru, P.K., Pegram, K.V., Kingston, A.C.N. and Rutowski, R.L. (2013) Male wing colour properties predict the size of nuptial gifts during mating in the Pipevine Swallowtail butterfly (*Battus philenor*). *Naturwissenschaften* **100**, p.507- 513.
- Ram, K.R., Ji, S. and Wolfner, M.F. (2005) Fates and targets of male accessory gland proteins in mated female *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology* **38**, p.1059- 1071.
- Ramm, S.A., Parker, G.A. and Stockley, P. (2005) Sperm competition and the evolution of the male reproductive anatomy in rodents. *Proceedings of the Royal Society London B: Biological Sciences* **272**, p.949- 955.

- Reddiex, A.J., Gosden, T.P., Bonduriansky, R. and Chenoweth, S.F. (2013) Sex-specific fitness consequences of nutrient intake and the evolvability of diet preferences. *The American Naturalist* **182**, p.91- 102.
- Reeson, A.F., Wilson, K., Gunn, A., Hails, R.S. and Goulson, D. (1998) Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. *Proceedings of the Royal Society of London B: Biological Sciences* **265**, p.1787- 1791.
- Reeve, J.P. and Fairbairn, D.J. (1999) Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* **83**, p.697- 706.
- Reid, J. (1976) Techniques. In *The Moths and Butterflies of Great Britain and Ireland*. Vol I (Ed. by J. Heath) p.117- 132. London: Current Press.
- Rice, W.R. and Chippindale, A.K. (2001) Intersexual ontogenetic conflict. *Journal of Evolutionary Biology* **14**, p.685- 693.
- Rigby, M.C. and Jokela, J. (2000) Predator avoidance and immune defence: costs and trade-offs in snails. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, p.171- 176.
- Robert, P. and Frerot, B. (1998) Oviposition behaviour of *Sesami nonagroides* Lefebvre (Lepidoptera: Noctuidae) – effect of conspecific eggs. *Annals of the Entomological Society of France* **34**, p.189- 194.
- Robinson, R. (1971) *Lepidoptera genetics*. Pergamon Press Ltd. Oxford.
- Rodd, H.F., Hughes, K.A., Grether, G.F. and Baril, C.T. (2002) A possible non-sexual origin of mate preference: are guppies mimicking fruit? *Proceedings of the Royal Society London B: Biological Sciences*. **269**, p.475- 481.
- Roeder, K.A. and Behmer, S.T. (2014) Lifetime consequences of food protein-carbohydrate content for an insect herbivore. *Functional Ecology* **28** (5), p.1135- 1143.

- Rolff, J. (2002) Bateman's principle and immunity. *Proceedings of the Royal Society of London B: Biological Sciences* **269**, p.867- 872.
- Rolff, J. and Siva-Jothy, M.T. (2002) Copulation corrupts immunity: a mechanism for a cost of mating in insects. *PNAS* **99** (15), p.9916- 9918.
- Rolff, J., Armitage, S.A.O. and Coltman, D.W. (2005) Genetic constraints and sexual dimorphism in immune defence. *Evolution* **59** (8), p.1844- 1850.
- Rothschild, M. (1978) Hell's angels. *Antenna* **2**, p.38- 39.
- Saejong, A., Tidbury, H., Siva-Jothy, M.T. and Boots, M. (2010). Examining the relationship between hemolymph phenoloxidase and resistance to a DNA virus, *Plodia interpunctella* granulosis virus (PiGV). *Journal of Insect Physiology* **56**, p.1232- 1236.
- Sait, S.M., Begon, M. and Thompson, D.J. (1994) The influence of larval age on the response of *Plodia interpunctella* to a granulosis virus. *Journal of Invertebrate Pathology* **63**, p.107- 110.
- Sait, S.M., Gage, M.J.G. and Cook, P.A. (1998) Effects of a fertility-reducing baculovirus on sperm numbers and sizes in the Indian meal moth, *Plodia interpunctella*. *Functional Ecology* **12**, p.56- 62.
- Sakaluk, S.K., Campbell, M.T., Clark, A.P., Johnson, J.C. and Keorpes, P.A. (2004) Hemolymph loss during nuptial feeding constrains male mating success in sagebrush crickets. *Behavioural Ecology* **15** (5), p.845- 849.
- Sathayon, R. and Couldridge, V.C.K. (2013) Female preference for blue in Japan blue guppies (*Poecilia reticulata*). *Environmental Biology of the Fish* **96**, p.953- 959.
- Schärer, L., Rowe, L. and Arnqvist, G. (2012) Anisogamy, chance and the evolution of the sex roles. *Trends in Ecology and Evolution* **27** (5), p.260- 264.
- Schmid-Hempel, P. (2003) Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society London B: Biological Sciences* **270**, p.357- 366.

- Schneider, J.M., Herberstein, M.E., De Crespigny, F.C., Ramamurthy, S. and Elgar, M.A. (2000) Sperm competition and small size advantage for males of the golden orb-web spider *Nephila edulis*. *Journal of Evolutionary Biology* **13**, p.939- 946.
- Shelby, K.S. and Popham, H.J.R. (2006) Plasma phenoloxidase of the larval tobacco budworm, *Heliothis virescens*, is virucidal. *Journal of Insect Science* **6** (13)
- Sheldon, B.C, and Verhurst, S. (1996). Ecological immunity: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11** (8), p.317- 321.
- Siberglied, R.E., Shephard, J.G. and Dickinson, J.L. (1984) Eunuchs: the role of apyrene sperm in the Lepidoptera? *The American Naturalist* **123** (2), p.255- 265.
- Simmons, L.W., Tomkins, J.L. and Hunt, J. (1999) Sperm competition games played by dimorphic male beetles. *Proceedings of the Royal Society London B: Biological Sciences* **266**, p.145- 150.
- Simmons, L.W. (2001) *Sperm competition and its evolutionary consequences in the insects*. Princeton, New Jersey: Princeton University Press.
- Simmons, L. W. (2005) The evolution of polyandry: sperm competition, sperm selection and offspring viability. *Annual Review of Ecology, Evolution, and Systematics* **36**, p.125-146.
- Simpson, S.J. and Abisgold, J.D. (1985) Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiological Entomology* **10**, p.443- 452.
- Simpson, S.J. and Raubenheimer, D. (1993) A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. *Philosophical Transactions of the Royal Society B: Biological Sciences* **342** (1302), p.381- 402.

- Simpson, S.J. and Raubenheimer, D. (2007) Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. *The Journal of Gerontology Series A: Biological Sciences and Medical Sciences* **62** (7), p.707-713.
- Simpson, S.J. and Raunbenheimer, D. (2009) Macronutrient balance and lifespan. *Aging* **1**, p.875- 880.
- Simpson, S.J. and Raubenheimer, D. (2012) *The nature of nutrition: a unifying framework from animal adaptation to human obesity*. Princeton, NJ: Princeton University Press.
- Smith, I.R.L. and Crook, N.E. (1988). *In vivo* isolation of baculovirus genotypes. *Virology* **166**, p.240- 244.
- Snook, R.R. (1997) Is the production of multiple sperm types adaptive? *Evolution* **51** (3), p.797- 808.
- Snook, R.R. (2005) Sperm competition: not playing by the numbers. *Trends in Ecology and Evolution* **20** (1), p.46- 53.
- South, S., House, C.M., Moore, A.J., Simpson, S.J. and Hunt, J. (2011) Male cockroaches prefer a high carbohydrate diet that makes them more attractive to females: implications for the study of condition dependence. *Evolution* **65**, p.1594- 1606.
- Stahlschmidt, Z.R., Rollinson, N., Acker, M. and Shelley, A.A. (2013) Are all eggs created equal? Food availability and the trade-off between reproduction and immunity. *Functional Ecology* **27**, p.800- 806.
- Svensson, E.I., McAdam, A.G. and Sinervo, B. (2009) Intralocus sexual conflict over immune defence, gender load, and sex-specific signalling in a natural lizard population. *Evolution* **63** (12) p.3124- 3135.

- Tammaru, T., Kaitaniemi, P. and Ruohomäki, K. (1996) Realized fecundity in *Epirrita autumnata* (Lepidoptera; Geometridae): relation to body size and consequences to population dynamics. *OIKOS* **77**, p.407- 416.
- Telang, A., Booton, V., Chapman, R.F. and Wheeler, D.E. (2001) How female caterpillars accumulate their nutrient reserves. *Journal of Insect Physiology* **47**, p.1055-1064.
- Telang, A., Buck, N.A., Chapman, R.F. and Wheeler, D.E. (2003) Sexual differences in postingestive processing of dietary protein and carbohydrate in caterpillars of two species. *Physiological and Biochemical Zoology* **76** (2), p.247- 255.
- Tigreros, N. (2013) Linking nutrition and sexual selection across life stages in a model butterfly system. *Functional Ecology* **27**, p.145- 154.
- Tigreros, N. and Lewis, S.M. (2011) Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, *Tribolium castaneum*. *Journal of Evolutionary Biology* **24**, p.835- 842.
- Tigreros, N., Sass, E.M. and Lewis, S.M. (2013) Sex-specific response to nutrient limitation and its effects on female mating success in a gift-giving butterfly. *Evolutionary Ecology* **27**, p.1145- 1158.
- Tisdale, R.A. and Sappington, T.W. (2001) Realized and potential fecundity, egg fertility and longevity of laboratory-reared female beet armyworm (Lepidoptera: Noctuidae) under different adult diet regimes. *Annals of the Entomological Society of America* **94** (3), p.415- 419.
- Tregenza, T. and Wedell, N. (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology* **9**, p.1013- 1027.
- Tregenza, T. and Wedell, N. (2002) Polyandrous females avoid costs of inbreeding. *Nature* **415** (3), p.71- 73.

- Triggs, A. and Knell, R.J. (2012) Interactions between environmental variables determine immunity in the Indian meal moth *Plodia interpunctella*. *Journal of Animal Ecology* **81**, p.386- 394.
- Trivers, R. (1972) Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man, 1871–1971* (pp. 136–179). Chicago: Aldine.
- Tuni, C., Beveridge, M. and Simmons, L.W. (2013) Female crickets assess relatedness during mate guarding and bias storage of sperm towards unrelated males. *Journal of Evolutionary Biology* **26**, p.1261- 1268.
- Uhl, G., Nessler, S.H. and Schneider, J.M. (2010) Securing paternity in spiders? A review on occurrence and effect of mating plugs and male genital mutilation. *Genetica* **138** (1), p.75- 104.
- Vahed, K. (1998) The function of nuptial feeding in insects: a review of empirical studies. *Biology Reviews* **73**, p.43- 78.
- Vahed, K. and Parker, D.J. (2012) The evolution of large testes: sperm competition or male mating rate? *Ethology* **118**, p.107- 117.
- Valtonen, T.M., Kangassalo, K., Pölkki, M. and Rantala, M.J. (2012) Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. *PLoS ONE* **7** (2), e31611.
- Van Doorn, G.S. (2009) Intralocus sexual conflict. *Annals of the New York Academy of Sciences* **1168**, p.52-71.
- van Maanen, M. J., Smeets, C. J. M. & Beijnen, J. H. (2000) Chemistry, pharmacology and pharmacokinetics of *N,N',N''*-triethylenethiophosphoramidate (ThioTEPA). *Cancer Treatment Reviews* **26**, p.257- 268.
- Voss, R. (1979) Male accessory glands and the evolution of copulatory plugs in rodents. *Occasional Papers of the Museum of Zoology* **689**, p.1- 28.

- Wade, M.J. and Schuster, S.M. (2004) Sexual selection: harem size and the variance in make reproductive success. *American Naturalist* **164**, p.83- 89.
- Watanabe, M., Bon'no, M. and Hachisuka, A. (2000) Eupyrene sperm migrates to spermatheca after apyrene sperm in the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae). *Journal of Ethology* **18**, p.91- 99.
- Wedell, N. (1997) Ejaculate size in bushcrickets: the importance of being large. *Journal of Evolutionary Biology* **10**, p.315- 325.
- Wedell, N. and Cook, P.A. (1999) Butterflies tailor their ejaculate in response to sperm competition risk and intensity. *Proceedings of the Royal Society London B: Biological Sciences* **266**, p.1033- 1039.
- Wedell, N., Gage, M.J.G. and Parker, G.A. (2002) Sperm competition, male prudence and sperm-limited females. *Trends in Ecology and Evolution* **17**, p.313- 320.
- Wedell, N. (2005) Female receptivity in butterflies and moths. *Journal of Experimental Biology* **208**, p.3433- 3440.
- Wedell, N. (2006) Male genotype affects females fitness in a paternally investing species. *Evolution* **60** (8), p.1638- 1645.
- Wedell, N., Kvarnemo, C., Lessells, M. and Tregenza, T. (2006) Sexual conflict and life histories. *Animal Behaviour* **71**, p.999- 1011.
- Wedell, N. (2010) Variation in male courtship costs in butterflies. *Behavioural Ecology and Sociobiology* **64**, p.1385- 1391.
- Weir, L.K., Grant, J.W.A. and Hutchings, J.A. (2011). The influence of operational sex ratio on the intensity of competition for mates. *American Naturalist* **177** (2), p.167- 176.
- Welke, K. and Schneider, J.M. (2009) Inbreeding avoidance through cryptic female choice in the cannibalistic orb-web spider *Argiope lobate*. *Behavioural Ecology*, arp097.

- Wheeler, D. (1996) The role of nourishment in oogenesis. *Annual Review of Entomology* **41**, p.407- 431.
- Wigby, S. and Chapman, T. (2004). Female resistance to male harm evolves in response to manipulations of sexual conflict. *Evolution* **58** (5), p.1028- 1037.
- Wigby, S. and Chapman, T. (2005) Sex peptide cause mating costs in female *Drosophila melanogaster*. *Current Biology* **15**, p.316- 321.
- Wigby, S., Sirot, L.K., Linklater, J.R., Buehner, N., Calboli, F.C.F., Bretman, A., Wolfner, M.F. and Chapman, T. (2009) Seminal fluid protein allocation and male reproductive success. *Current Biology* **19** (9), p. 751- 757.
- Williams, G.C. (1964) The life-history of the Indian meal-moth, *Plodia interpunctella* (Hübner) (Lep Phycitidae) in a warehouse in Britain and on different foods. *Annals of Applied Biology* **53**, p.459- 475.
- Wilson, K., Thomas, M.B., Blandford, S., Doggett, M., Simpson, S.J. and Moore, S.L. (2002) Coping with crowds: density-dependant disease resistance in desert locusts. *Proceedings of the National Academy of Sciences* **99** (8), p.5471- 5475.
- Winkler, J.D., Stölting, K.N., and Wilson, A.B. (2012) Sex-specific responses to fecundity selection in the broad-nosed pipefish. *Evolutionary Ecology* **26**, p.701- 714.
- Wit, J., Sarup, P., Lupsa, N., Malte, H., Frydenburg, J. and Loeschoke, V. (2013) Longevity for free? Increased reproduction with limited trade-offs in *Drosophila melanogaster* selected for increased lifespan. *Experimental Gerontology* **48**, p.349- 357.
- Xu, J. and Wang, Q. (2009) A polyandrous female moth discriminates against previous mates to gain genetic diversity. *Animal Behaviour* **78**, p.1309- 1315.

- Xu, J. & Wang, Q. (2010a) Thiotepe, a reliable marker for sperm precedence measurement in a polyandrous moth. *Journal of Insect Physiology* **56**, p.102-106.
- Xu, J. & Wang, Q. (2010b) Mechanisms of last male precedence in a moth: sperm displacement at ejaculation and storage sites. *Behavioural Ecology* **21**, p.714-721.
- Xu, J. and Wang, Q. (2013) Trade-off between adult body size and juvenile survival: an experimental test of parental effects in the Mediterranean flour moth. *Australian Journal of Entomology* **52**, p.403- 406.
- Yeates, S.E. Diamond, S.E., Einum, S., Emerson, B.C., Holt, W.V. and Gage, M.J.G. (2013) Cryptic choice of conspecific sperm controlled by the impact of ovarian fluid on sperm swimming behaviour. *Evolution* **67** (12), p.3523- 3536.
- Zeh, J.A and Zeh, D.W. (1996) The evolution of polyandry I: Intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society of London S: Biological Sciences* **263**, p.1711- 1717.
- Zeh, J.A. and Zeh, D.W. (1997) The evolution of polyandry 2: Post-copulatory defences against genetic incompatibility. *Proceedings of the Royal Society London B: Biological Sciences* **264**, p.69- 75.
- Zera, A.J. and Harshman, L.G. (2001) The physiology of life-history trade-offs in animals. *Annual Review of Ecology and Systematics* **32**, p.95- 126.
- Zuk, M and Stoehr, A.M. (2002) Immune defence and host life-history. *American Naturalist* **160**, p.9- 22.